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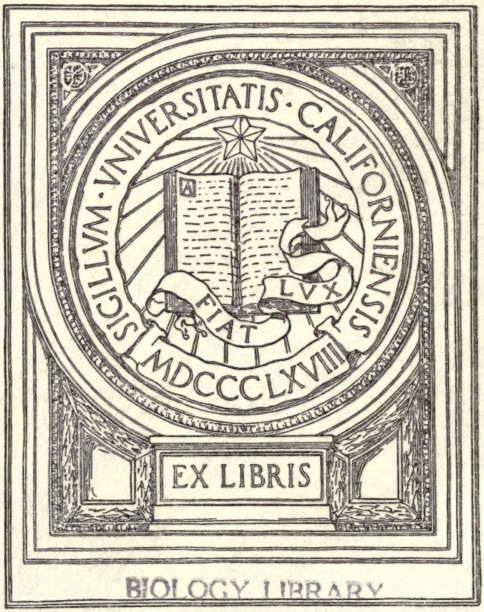

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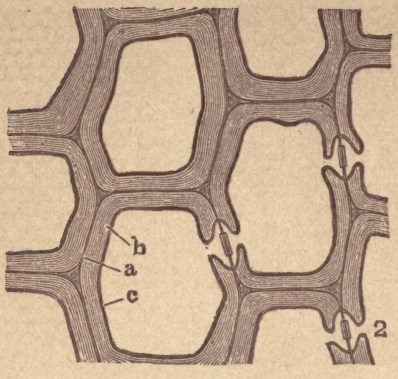

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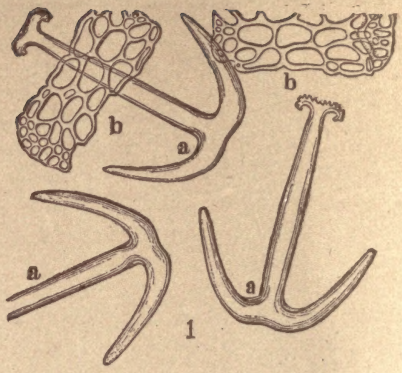

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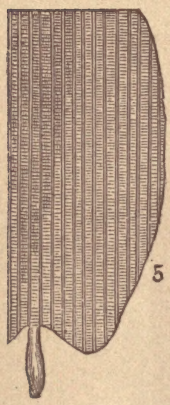

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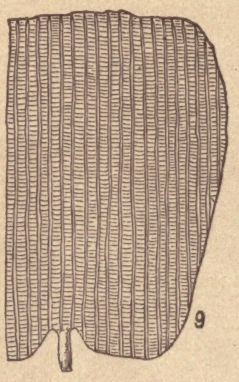

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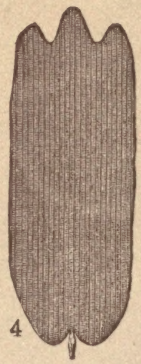

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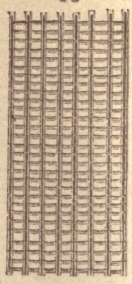

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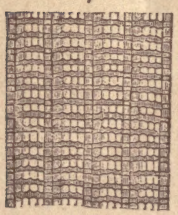

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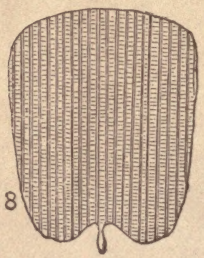

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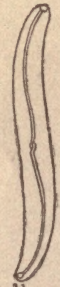

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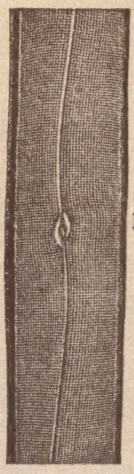

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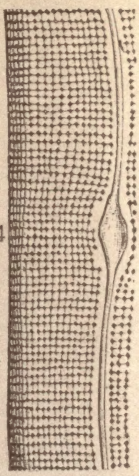

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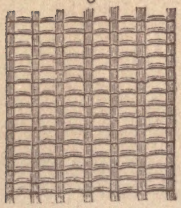

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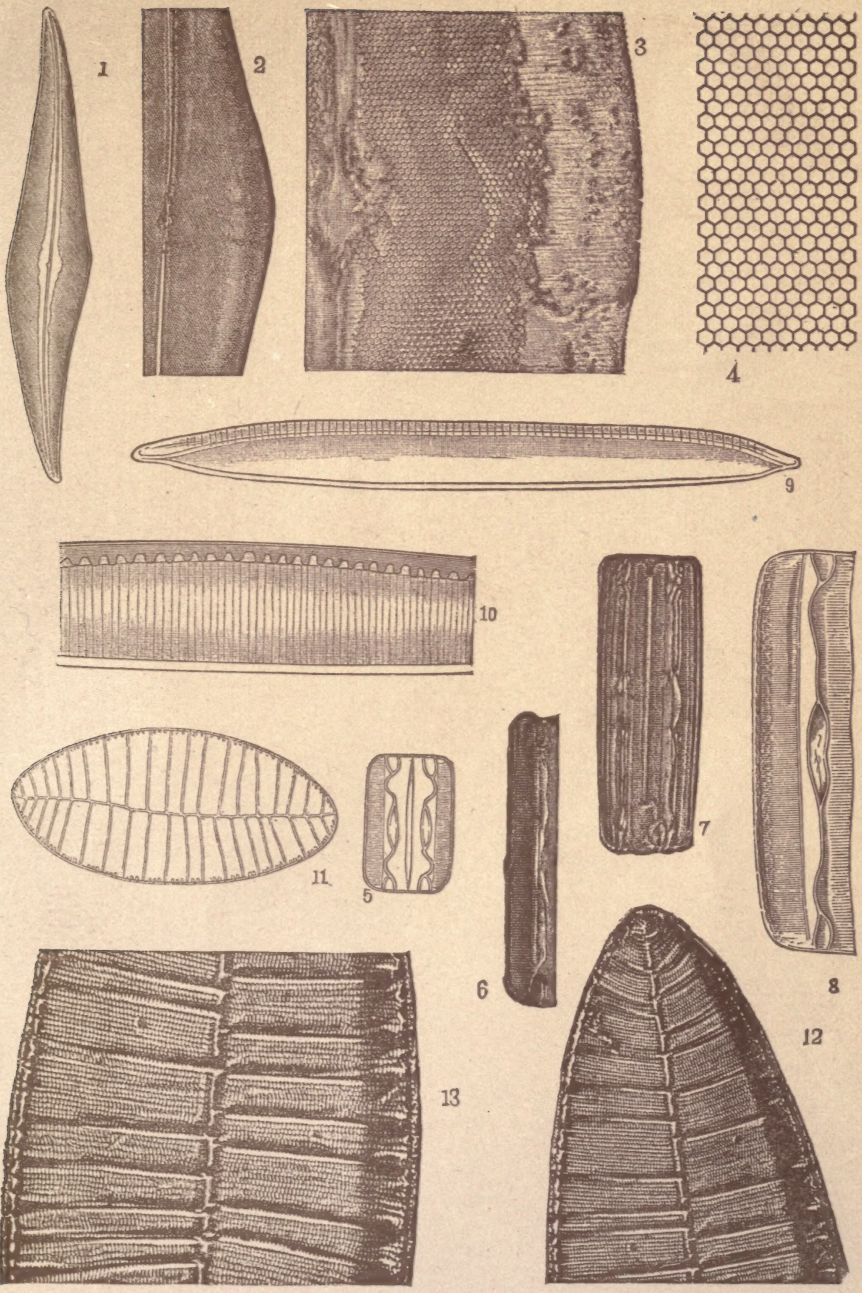

TEST OBJECTS.

UNIV. OF

The Microscope in Botany

A GUIDE

FOR THE

MICROSCOPICAL INVESTIGATION

OF

VEGETABLE SUBSTANCES

FROM THE GERMAN OF

DR. JULIUS WILHELM BEHRENS

TRANSLATED AND EDITED BY

REV. A. B. HERVEY, A.M.

ASSISTED BY

R. H. WARD, M.D., F.R.M.S.

ILLUSTRATED

WITH THIRTEEN PLATES AND ONE HUNDRED AND FIFTY-THREE CUTS

Boston

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1885

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AUTHOR'S PREFACE.

THE preparation of this work has engaged my time for several years. In the beginning of 1880, I finally concluded to work up for publication the material relating to the microscopical investigation of vegetable substances, which I had previously collected for private use. Several friendly botanists, to whom I communicated my designs, counseled me very earnestly to carry them out. As the publishers also were prepared to undertake the work at once, the preparation of the manuscript and the printing of it have gone on simultaneously since about Easter, 1880.

For a work to be useful in those microscopical inquiries which are most important in the botanical laboratory, it need teach neither optics nor histology.

The student will, therefore, find in the work before him a brief description only, of the microscopical apparatus applicable to his uses (Chapters I and II), together with directions for its use. If he wishes to become acquainted with the instrument from the standpoint of the optical physicist he must go to the larger manuals of Hartig, of Nägeli and Schwendener, or the shortly to be published hand-book of Dippel and Abbe. These are works which, if studied with the necessary care, will furnish a very perfect understanding of the performance of the microscopical apparatus, but which on account of their lengthy theoretical analyses are but poorly adapted for the table of the practical microscopist.

The first and second chapters treat of the microscope and its accessory apparatus, while the third contains directions for the preparation of microscopic specimens. Every one knows that the preparation of specimens cannot be learned by the mere

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reading of these detailed statements. In this matter manual instruction is the main thing. However, the study of this chapter will open in many places new points of view to the young microscopist, and give him occasion here and there independently to apply new methods.

I have to thank my friend, Dr. Conwentz, for kindly undertaking the preparation of the section relating to fossil plants.

The most important part of the whole work is in the fourth and fifth chapters. They contain what has heretofore — incorrectly — been called micro-chemistry. The fourth chapter treats of microscopical reagents and the fifth of the microscopical investigation of vegetable substances.

Until the middle of 1880, there was no useful compilation of the matters pertaining to this subject which was at all abreast with the science of the day in existence. But in the meantime the "Botanical Micro-chemistry" of Poulsen, a brief compilation of the methods of micro-chemical reactions, has appeared. I believe, however, that notwithstanding this little book has justly had a wide circulation, the value of the corresponding chapters of the present work will not be materially lessened.

Poulsen's work is designed mainly for beginners and therefore contains only the most important methods of reaction in the barest outline. On the other hand, the chapter of this work which deals with the microscopical investigation of vegetable substances, furnishes an *exhaustive* treatment of these matters, and at the same time is so arranged as to make the specialist quite independent of the widely dispersed literature of the subject which is often hidden and not seldom difficult to obtain. But at the same time, a compilation of the literature, as complete as possible, greatly facilitates reference to the original works. It is evident that the point of view thus briefly outlined must require a handling of the case fundamentally different from that which Poulsen has given it. I have kept the chemical (*i. e.*, the physiologico-chemical) point of view in the foreground throughout, not only in the arrangement of the whole, but also in the management of each separate section. The arrangement of the subject matter follows closely that of the

new edition of Husemann and Hilger's "Vegetable Substances," ("Pflanzenstoffe") which I regret to say is not yet completed. Thus the use of that work in connection with my compilation is made more convenient. I am firmly convinced that the micro-chemist will have many interesting outlooks opened to him, and many new methods suggested by the study of Husemann and Hilger's work. It seems to me that the separate microscopical investigation of vegetable substances is the only way (leading out from their chemical qualities) to attain a true comprehension of the methods of microscopical research.

The discerning reader will soon discover that the whole chapter is by no means a mere compilation, but that I have critically sifted the existing materials. The useless I have rejected. The useful, however, I have taken not altogether on trust and faith, but as far as possible carefully tested. Indeed, I have tested everything it was possible to in the nature of things, and this experimentation has already consumed more than three years of working time.

For the presentation of the whole I have chosen — as brevity also seemed to require — a purely objective form. Subjective views are kept entirely in the background, and at no time have I entered into argument. Various new discoveries, the results of my experimentations, will be published later in a separate monograph.

In the compilation of the literature I have attempted the utmost possible completeness. For my success in this, I am mainly indebted to the University library, which lacks scarcely a single treatise of all the literature quoted. With hardly a noteworthy exception, I have seen and read it all.

But a small portion of the illustrations for this work are copies. Much the greater part are original drawings which I have for the most part made upon wood myself. So far as this remark applies to the microscopical apparatus, they have been photographed under my directions with the use of a very small diaphragm; they appeared almost black, but for engraving, I finished the pictures, which had been photographically transferred to the wood, in lines with sepia or India ink and white.

Since the printing went on continuously with the elaboration of the manuscript, I could not in some sections refer to the very latest literature, as, for example, in Cellulose, Strasburger's beautiful work on the structure and growth of the cell wall could not be cited, likewise some recent monographs concerning the structure of the nucleus. But as far as it was possible I have cited the very latest literature.

I shall be much obliged to those gentlemen who may give the fifth chapter critical attention if they will communicate to me any accidental omission or error.

W. BEHRENS.

Göttingen, Dec. 18, 1882.

TRANSLATOR'S PREFACE.

In presenting to the English-speaking public a translation of Dr. Behrens' invaluable work, a few explanatory words seem to be needed.

It is the first purpose of this work to guide students in all those inquiries relating to the physical products of cell-life in plants, which may be conducted under the microscope, by means of chemical and other reactions. It undertakes so to instruct him that he can make a thin section of any part or organ of a plant, and, putting it under his lens, answer to himself the questions: What have the life processes thus far produced here, and what are they now producing?

It deals with the anatomical constitution of the cell, and of plant tissue, and yet, its inquiries relate far more to physiological and biological processes and results than to matters purely anatomical and histological. The unit of life is the cell. The physical embodiment of that life is the protoplasm, the physiological center of which is the nucleus. The formed matter, the finished product of life, is the cell wall and some cell contents, and in the tissue the middle lamella also. The life of different cells and its embodiment are absolutely indistinguishable. The protoplasm and nucleus of, for example, wood-forming cells and cork-forming cells are microscopically and chemically alike. Differentiation appears only in the finished product of the life process, viz.: in some cell-contents, and mainly in the cell wall. It is to the nature of this, therefore, largely, in all the various kinds of vegetable tissue that our inquiries relate. But not alone to this: for a full investigation of nearly all the other elements of plant life is carefully marked out,—of functional protoplasm and reserve protoplasm or proteid matter, of starch, chlorophyll, sugar, etc., elements so largely concerned in the life processes.

The treatise occupies a field almost entirely to itself in the botanical literature both of Germany, and now of the English-

speaking world. It is sincerely hoped that its publication in this form will stimulate in this country investigations into the deeper problems of plant life. It will be seen by reference to the literature cited, that there is an open field for American botanists, since the works referred to almost exclusively embody the results of German research, while a few are of French origin, fewer still of English and none whatever of American.

I alone am responsible for the translation, and have endeavored to take a medium course in it, following the text neither too literally, nor yet translating so freely as to introduce shades of meaning not in the author's mind. I am inclined to believe my errors are those more often leaning to the former than to the latter side. In that direction, I suppose, lie the temptations for the conscientious translator, though in the interests of a good style he would be more readily pardoned for sinning on the other side.

Early in the enterprise, Dr. R. H. Ward very kindly consented to undertake the revision of the two chapters which deal with the microscope and its accessories. He states the plan upon which he has made the somewhat extensive changes in these chapters as follows:—

"The changes in Chapters I and II consist wholly in the omission of illustrations and descriptions of apparatus in the Continental style, which is comparatively unused and unavailable here, and the substitution of American forms. It was desired, and deemed necessary, that a work intended as a practical handbook should describe and discuss such instruments as are likely to be most generally preferred and used by the majority of its readers. The author's full and very valuable discussions on the methods of work, and on the construction, testing, care and use of the optical parts, and of the most important accessories, are retained without abridgment or material amendment.

"The construction of the Stand is illustrated by several models, in order to exhibit the most common varieties believed to be eligible for such work, and (incidentally) to establish, by comparison of characteristic stands by prominent American makers, the claim to the existence of a new, serviceable and American type in addition to the two styles, Continental and English,

heretofore recognized. It will be noticed that the American type, as illustrated by Plates III to VI, VIIIa, and IX to XI, is intermediate in size and complexity between the other two, but built upon a radically different model from either; having much of the simplicity and portability of the Continental with much of the efficiency and versatility of the English style."

The changes in the third, fourth and fifth chapters, for which I alone am responsible, consist almost entirely of additions to the text. They relate in the third chapter exclusively to those methods, tools, materials, etc., which experience has taught American investigators to consider important in their work. The few additions to the remaining chapters relate only to those researches in this field, which, up to the beginning of 1884, and subsequent to the close of the author's work, had come under my notice.

The matter introduced into the text by the American editors is inclosed in brackets [ ] and usually signed with the respective editors' initials. Foot-notes by either of the editors are likewise mostly signed, and are referred to in the text by symbols, while those of the author are all numbered.

We are indebted to various firms of opticians, as well as to other parties, for the use of electrotypes, for which we here desire to express our cordial thanks.

The work of translating and editing this treatise has been done in the midst of the engagements of a busy professional life, in hours snatched at irregular intervals from the demands of pressing public and domestic cares; yet I have verified by actual experiment the larger part of all the statements and methods given by the author.

A. B. Hervey.

Taunton, Mass., Feb. 26, 1885.

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CHAPTER I.

THE MICROSCOPE.

I. Introduction.

The microscope, as an instrument of observation, holds at the present time the most prominent position in the department of scientific botany, especially in vegetable anatomy and to some extent also in physiology. Vegetable anatomy treats entirely of the investigation of the constituent elements of vegetable organisms, and the minuteness of these parts makes the use of the instrument in question almost always necessary The cultivation of vegetable anatomy has been so far dependent upon the development of the microscope that those periods in which most improvements have been made in it correspond in many cases to those in which the principal development of this science has occurred. On the other hand also, the wider development of phytotomy has not been without its influence upon the improvement of certain parts of the microscope.

The name of the apparatus as well as its invention belongs to the later middle ages, somewhere in the fifteenth or sixteenth century. The instrument was called the microscope (*μικρος*, small, and *σκοπεω*, to look upon, to observe by means of the sight) because it allowed very small objects, grains of sand, insects and the like to be seen magnified. One must not think, however, that those first very timid attempts to construct magnifying glasses can in any way be compared with the instruments fabricated in recent times. The first of these productions were the "flea-glasses," *Vitra pulicaria* and

muscaria, which served the learned men of that time more as a "highly curious and frightful microscopical amusement for eyes and mind" than for scientific observations. They consisted of the kind which we see to-day in our toy shops, and were made of a single glass lens which was the segment of a sphere of small diameter. This lens was fastened into a wooden tube which bore at its lower end in the focus of the lens a small glass plate on which a crushed flea, a gnat, a fly's leg, or a like object was pasted. The "flea-glass" belonged to the indispensable requisites of a learned man of that time. It magnified about six to ten times, equivalent to an average magnifying glass of to-day. It makes the most comical impression on one to-day to read the descriptions which these Faustian *savants* give of their observations with the "flea-glasses." Many, amazed, described real monsters which they thus observed, while the less expert believed they saw the Devil himself in the innocent instrument.

Leeuwenhoek (1632–1723) one of the first who really set about scientific observations with the microscope, a man who, by his discovery of the infusoria, is known in the widest circles, used a simple microscope exclusively in his observations, but which, measured by the ideas of his time, possessed very strong magnifying powers. He understood how to grind very perfect little lenses with short focal distance, which he fastened between two right-angled plates of silver or brass (from 4 to 5cm. long, and about 3cm. broad) screwed upon each other in such a way that one could look down through two corresponding holes in the plates and through the lens lying between. Behind this mounted lens was a small pointed instrument capable of movement in all directions, on the end of which the object to be observed was impaled. The whole instrument was then held towards the light and the lens brought as near to the eye as possible. Leeuwenhoek's microscopes magnified from 40 to 100 times, a few 150, and one even 270 times.

But already before the time of Leeuwenhoek, that is, still before the close of the sixteenth century, or perhaps in the beginning of the seventeenth century, the *compound* microscope had been invented. It is very essentially distinguished from

the one already mentioned by this : that a picture is produced by the lens nearest the object, the so-called "objective," and that this image is viewed through a lens placed near the eye, the "ocular," relatively magnified. This principle is fundamental in all microscopes, in those also of the present time, though they may be constructed never so differently from those of the first inventor.

Who should be regarded as the inventor of the first compound microscope was for a long time a doubtful and frequently disputed question. According to the literary studies of Harting,[1] it appears pretty nearly beyond doubt that this merit should be ascribed to two spectacle makers in Middelburg in Holland, Hans & Zacharias Janssen, father and son. Previously Fontana, Galileo Galilei, and the Netherlander Drebbel also had been regarded as the first inventors of our instrument. In a work by Borel, a Frenchman, is a letter from a countryman and friend of the younger Janssen who among other things describes the first microscope in the following way.[2] "It possessed not, as such an instrument would now, a short tube, but one almost a foot and a half long. The tube itself was of gilded brass and was fixed in the middle at a height of two fingers on three bronze-crested dolphins. The foot consisted of a disk of ebony that carried various small instruments and minute objects which we viewed from above in almost miraculously magnified form."

Although with the invention of the compound microscope in general, there had been worked out the proper arrangement which should be given to the apparatus, still the construction of magnifying glasses during the century succeeding the invention was so faulty that it was little or not at all fitted for making those observations which have been attained in recent times.

During that period the object under investigation was viewed with reflected light only, it being concentrated upon the object from the lamp which was the source of light, by means of a

[1] Harting. Das Microskope Braunschweig, 1859, pp. 586–596.

[2] The Latin text is printed in Harting, *l. c.*, p. 589. The work of Borel bears the title "De vero telescopii inventore, cum brevi omnium conspiciliorum historia. Accedit etiam centuria observationum microscopicarum. Hag. Comitum, 1655.

globe filled with water, or by a condensing lens. Such an instrument adapted only for superficial illumination is illustrated, for example, by Robert Hooke,[3] who also states that a thin section through a flask cork, on a dark background, looks like a honey comb, in which could be distinguished open spaces (pores) and separating walls.[4] The illuminating reflector (mirror) which is indispensable to us, and which we place under the object, in order to view it with transmitted light was first introduced for the use of the compound microscope about the year 1735, by Culpeper and Scarlet,[5] and a few years later (1740) by Wilson,[6] for the simple microscope. Thus an important step forward was made.

But at that time, the construction and combination of lenses for the compound microscope were very faulty. One did not then as now employ an ocular, made of two glasses, and an objective of several lenses, but each consisted at that time of but *one* glass. The consequence was that the microscopic image often appeared very much bent, because only its middle portions were clearly shown, while the edges were distorted beyond recognition. This explains what Wolf[7] expressly declared, in the year 1723, that at that time the simple microscope was much more in use than the compound (see above, Leeuwenhoek) and that one would much rather use the former, especially in high magnifications, than the latter.

From the following, it becomes clear how these microscopes gradually gave place to those with flat fields of view. The instruments of Drebbel, Galilei and probably also the one of Janssen, above described, possessed, as remarked, two convex lenses, the one serving as ocular, and the other as objective. Fontana inserted in his microscope an intermediate concave glass between the other two lenses. Hook did the same, but principally with a view to enlarge the field of vision, and he let it remain when he saw the image clearly and distinctly. A further step was made towards this improvement by Divini, about 1670, who first united two plano-convex lenses in one ocular,

[3] R. Hooke, Micrographia, or some physiological descriptions of minute bodies made by magnifying glasses. London. 1667.

[4] J. Sachs, Geschichte der Botanik. p. 247. [5] Harting, *l. c.*, p. 672.

[6] Harting, *l. c.*, p. 615. [7] Sachs, *l. c.*, p. 267.—See also, Harting, *l. c.*, p. 688.

as it is done to-day. Soon after that, doublets also, which had already been in use for some time as simple microscopes, began to be employed as objectives. At last, toward the end of the seventeenth century, there came in combinations of lenses as objectives. They were either biconvex or plano-convex[8] lenses of unlike foci, permitting therefore by different combinations the production of lower or higher magnifications, while in the oldest compound microscopes the different magnifications had been brought about in this way, the tube was constructed like that of a telescope of three or four parts which slid into each other, and the magnifying power was increased by drawing these out.

But the power of the microscope was so small that a scholar of that time praised a microscope which magnified eighty diameters, as being "certainly quite gigantic in its magnification" (*quod certe insigne augmentum est*).

While we have seen that in the fabrication of the microscope it has gradually come about that the spherical aberration has in great part been overcome by means of suitable forms and combinations of lenses, it was not until the concluding third of the last century that another great defect of the instrument was removed, namely, the chromatic aberration. Newton had indeed already, towards the end of the seventeenth century, expressed the opinion that glasses might be constructed which should produce a colorless image by a combination of two lenses, made of material having the greatest possible likeness of refractive power, and the greatest possible difference in color-dispersing power, although it was not possible for him to bring the matter to experimental proof.[9] Dollond had indeed, in 1758, constructed the first achromatic telescope, and so had reduced that theoretical calculation to practice. Euler also, in 1771, had established a coherent theory of Achromatics, and Fuss in 1774, in accordance with the analyses of the latter, gave directions for con-

[8] While it is naturally impossible to correct the spherical aberration by a combination of biconvex glasses, it is quite easily done by the use of plano-convex lenses, whose plane side is turned under and brought next to the object. Although toward the end of the last century plano-convex object-lenses were generally used, they were constantly so placed that the convex side was turned toward the object. Chevalier and Amici first used them reversed, and so reduced the spherical aberration to a minimum.

[9] Through inexact observation he was led into the error of supposing that materials, with nearly the same refractive index and very different color-dispersing power, did not exist.

structing an achromatic microscope. But it was held to be impossible to construct achromatic glasses of the small size required in microscopic objective lenses, and it was believed therefore that a microscope could never be made which would give at the same time in some measure high magnification and a clear sharp image.

According to Harting,[10] it was again a Hollander, van Deyl, (1807), who first constructed a really achromatic microscope, forming his objective out of two biconvex crown-glass lenses, and a biconcave flint-glass lens lying between. His instrument, however, for reasons stated in note 8, p. 5, possessed a very considerable spherical aberration.

A short time after that, Brewster made the attempt to substitute a fluid for the flint-glass middle lens (Newton had attempted to do the same thing already). It is known under the name of Brewster's achromatic globe. It is a glass globe filled with water in which two biconvex lenses are made to lie pole to pole in the optical axis. The contrivance has however never come into use.

After achromatic objective lenses for microscopes had been prepared by Fraunhofer, the most gifted optician of all time, there appeared in France, Chevalier (about 1824), and in Italy, Amici (1827 and later), who constructed objectives such as to-day are generally in use, under the designation of "aplanatic."[11] In them the spherical, as well as the chromatic aberration, was so far suppressed that they no longer essentially hindered microscopical observation. We shall become more intimately acquainted with the aplanatic lens in the subsequent portions of this chapter.

The names which in more recent times have had most significance in microscope-making, and with which, together with their contributions we shall become more intimately acquainted, in the proper place hereafter, are principally, Hugo von Mohl, Oberhäuser, Hartnack, Nachet, Merz, Plössl, Bénèche, Wasserlein, Pritchard, Ross, Zeiss, Seibert, Krafft, Winkel, Schieck, Leitz, Powell and others.*

[10] Harting, *l. c.*, p 691.

[11] From ἀ privative and πλαναω, to deceive, to lead astray.

*Certainly the names of our two greatest American opticians, Spencer and Tolles, should not be omitted from this list. A. B. H.

In our historical review, we have thus far drawn attention only to the optical part of the microscope, while the bearer of these parts, the stand, has been left out of sight.

In the period directly after the invention of the instrument, very little attention was devoted to the microscope stand. It commonly consisted of an elaborately turned piece of wood, while the cylindrical draw-tube was often made from pasteboard. The first important improvement which the mounted compound microscope received was the introduction of the illuminating mirror by Culpeper and Scarlet, as already noticed above. The microscopist now began to work with transmitted light. The object for observation was placed upon a perforated plate, the stage, which occupied a position between the mirror and the objective.

In order to focus the object, that is to say, to bring it exactly in the focal point of the object-glass, we may proceed in either of two ways, viz., the object table or stage may be made fast, and the adjustment produced by moving the tube which carries the optical apparatus towards it, or on the other hand the tube may be fixed and a vertical movement may be given to the stage. In the first compound microscopes with illuminating mirrors, from the factory of Culpeper and Scarlet, the stage was fixed, and the focussing was done by shoving the tube by hand. But already, about the middle of the eighteenth century, Cuff had employed the setting screw to accomplish a more exact focussing. In his microscopes the tube was fastened to a metal hinge which could move up and down on a perpendicular metal rod. By this manipulation, the approximate, or so-called, coarse adjustment was accomplished, while by a clamping screw, the hinge was made fast, and a very small shortening or lengthening of the hinge itself was accomplished by a second screw (the so-called micrometer screw) thereby making the fine adjustment. In later microscopes (of Martin, Jones, van Deyl, etc.) the tube was moved by a simple rack and pinion.

In the instruments of Chevalier the opposite plan was adopted, the stage was moved by means of a hinge, with a clamp screw on a vertical prismatic metal rod. The fine adjustment was produced, as in the instruments of Jones, by means of a carefully cut, small thread, fine-adjustment screw.

Afterwards these last contrivances were generally abandoned; only the microscopes of Amici now have them, and in the oldest stands of Plössl both the tube and the stage are movable on the same vertical rod.

In the instruments of the present time (or at least on the larger and medium stands), without exception, the adjustment is produced by moving the tube vertically in a direction perpendicular to the stage, which is made fast to the rod. The coarse adjustment is produced directly by free hand or by means of a rack and pinion. The fine adjustment is made by a fine screw.

While the stands of the first microscopes, which were made throughout of polished or unpolished wood, could satisfy but very modest demands as to their outward appearance, stands made of brass came into very general use at the end of the last century. There came a time when the outside appearance of the stand received by far the most attention, while the optical part was very little improved. There was brought into use with the stand also, at that time, every possible useless accessory (the so-called microscopical accessory apparatus) so that the observer was often more hindered than helped by it in his work.[12]

It was the famous phytotomist Hugo von Mohl who first expressly demanded the simplest constructed microscopes, and rebuked with sharp words the coquettish toying with stands which was becoming the fashion. He speaks about it,[13] for example, as follows. "The simpler the construction of the microscope is, the more easily and more quickly will one accomplish all the necessary movements. The more complicated the construction the more will they cost in time and reflection, and the more will the attention be distracted thereby during the observation. Whoever has not the manual dexterity to work with a simply constructed microscope, and finds it necessary to use a screw

[12] The author has recently had an opportunity to take a look at such an English instrument in the physical collection of the technical high school in Brunswick, of which one literally cannot get at the stage on account of screws, magnifying glasses and other things, and which one can recognize as a microscope generally only after the most exact consideration.

[13] H. v. Mohl, Mikrographie, Tübingen, 1846, p. 89.

instead of his fingers for every movement, is on that account disqualified for a microscopical observer, for he will labor in vain to prepare a usable specimen."

*

* *

After this short historical survey of the invention of the microscope, we will now turn to a consideration of the instrument itself. Since we shall assume that those general laws of optics concerning the refraction of rays of light, which may be found stated in every text-book are already known, it will be our aim in the following treatise to give a representation of the microscope without admitting special theoretical deductions. Afterwards the reader will be made acquainted with the methods of preparing botanical microscopic specimens, and at last there will be shown to him how their methodical investigation should be conducted.

But it will not perhaps be useless to insert here first of all the following general remarks.

From the start it must be clearly understood that the microscope is not an instrument to which one only needs to turn and look in, in order to behold some great discovery. The microscope is, on the contrary, an instrument whose use and management must be learned, but which then, if it be used with understanding and with regard for the most careful precautions, permits things to be seen which would be forever shut out from the unaided eye.

Under the microscope, however, we see always only a very small part of a natural body, and what is more important, we see that only in two dimensions, namely, in length and breadth. We can never at the same time perceive its thickness. We must therefore, in order to come to a clear conception of the microscopical structure of organs having a corporeal appearance, to the naked eye, contemplate different sections through it, made in the direction of the three dimensions of space, and then combine these by means of our mental eye. Thus there is required for seeing and understanding the microscopic image, not only activity of sense but activity of mind, also. I quote here, in reference to this, the declaration of one of the most highly

accomplished of living observers, Julius Sachs, as he writes in the History of Botany:[14] "Seeing is an art which must be learned and cultivated, a definite purpose must stimulate the will of the observer, to will to see exactly and rightly, to distinguish and combine what is seen." . . . . "By the invention of the microscope the eye became capable not merely of seeing small things larger, and in general of seeing the invisibly small, but much more; there was combined with the use of magnifying glasses the one other advantage, viz.: that then first we learned in general to see exactly and scientifically. In that we armed the eye with a magnifying glass, the attention was concentrated on a single point of the object, the seeing was in part indistinct and always of but a small part of the whole object. The perception of the visual nerve must be accompanied with a purposeful and intense reflection, in order to make the object observed in fragments by the magnifying glass, clear, in its inner connections, to the mental eye. So the eye, by being armed with the microscope, became itself a scientific instrument, which no longer ran over the objects with thoughtless movements, but received strict discipline from the understanding of the observer, and was kept to methodical work." . . . . . "As in every other science, so in the investigation of the structure of plants, the sense perception must be worked over by the understanding, to distinguish the important from the unimportant, and to bring the single perceptions into logical coherence, to follow a purpose in the investigation, but this purpose can be none other in the last instance for the plant anatomist, than that the whole inner structure of the plant in its collective coherency shall be so clearly comprehended that it may at any time, in all its details, be perfectly reproduced in perfectly sensible definiteness from the imagination. To attain this is not easy, because the more powerfully the microscope magnifies the smaller the part of the whole which it shows. Skilful and superior preparations, careful combinations of different images, and long practice are necessary to attain that object. The history of vegetable anatomy shows how difficult it has been for observers to gradually form a clear, coherent conception from these fragmentary views."

[14] Sachs, *l. c.*, p. 236, ff.

But there is also, for those who have already attained some facility in the use of the microscope, one important aid which is indispensable to methodical observation. Every one knows, and has often been compelled to make the remark to himself, that our memory can be said to be trustworthy only to a limited extent, and that the subjective impression which the brain receives is but imperfectly and often but temporarily fixed. It is especially necessary to resort to the help of the graphic art if one would preserve in the memory, with any exactness, a series of microscopical observations. This may be done in either of two ways. In the first place by keeping an exact record of the observations, and in the second place by endeavoring to fix the microscopic image on paper by means of pencil or brush, in other words by drawing it. The latter presupposes a real manual dexterity, a certain *technique*. But it will not be difficult for microscopists who are but little practised in drawing to acquire the necessary skill for this purpose. Besides this there is, as we shall show farther on, abundant and manifold apparatus, which allows the microscopic image to be thrown down by means of a reflecting prism upon a sheet of paper lying near the microscope, where it may be traced with the pencil without further trouble. But these contrivances are to be used, especially by the beginner, only with the greatest care. For the microscopical drawing should not be merely the spiritless crude copy of the image seen, but it should receive into itself all the experiments, all the studies which the observer has made upon the object; in a word it should be idealized. In connection with the opinion just now expressed I will quote a restrictive remark of Sachs: "A microscopical drawing, like illustrations of objects of natural science generally, cannot quite lay claim to replace the object itself; all the more then should it repeat with all distinctness what the observer has perceived and in so far support the description in the text. The drawing will be all the more perfect, the more the eye is trained in observing and the understanding in interpreting the forms. The illustrations should show to the reader nothing other than what has been traversed throughout by the mind of the observer, for only so can it serve to bring the two to a mutual understand-

ing. But the case has still another significance: even during the drawing of a microscopic object it is necessary for the eye to dwell on single lines and points, in order to comprehend their true dependence in respect to all the dimensions of space; it often happens thence that relations are perceived, which, previously, even in the most careful observation, were not noticed; for, however the question being investigated is determined, new questions are opened. Thus as the eye is first, by the use of the microscope, trained to scientific seeing, so first by careful drawing of the object will the educated eye become a growing councillor of the investigating mind."[15]

One already skilled in drawing, when he begins to make microscopical observations and drawings, will at first produce quite imperfect pictorial representations; but by the constant use and practice of the eye in microscopical seeing, the drawings will tend to ever greater perfection and instructiveness.

Much has been written as to the personal characteristics which the microscopist should possess. We limit ourselves, therefore, under the supposition of making a theoretical presentation, to the four following factors required by him: a skilful hand, good eyes, a tranquil mind, and self knowledge.

For the preparation of microscopical specimens, which presupposes the careful management of the most various tools, a certain skilfulness of hands is one of the first conditions. In relation to the eyes, the short-sightedness so common to scientifically educated people, is in no way a hindrance to microscopical observation. On the contrary, it is often very useful in the preparation of specimens, as a brilliant example may be quoted to show, in the case of one of the most accomplished microscopists and vegetable anatomists, Wilhelm Hofmeister, who, in preparing objects, brings them very close before his unspectacled, very shortsighted eyes, and in this way sees relatively very large, thus using the eyes in place of a mounting microscope.[16] Observe with the right eye, but one should avoid

[15] Sachs, *l. c.*, p. 280.

[16] Spectacle wearers do best when they remove the glasses during exact microscopical observation, and they should be used in preparing microscopical drawings, only by extremely shortsighted people, when it cannot be done without them. In this manipulation there frequently results (at least to the shortsighted author) this discomfort: since the right

pinching up the left eye meanwhile, because the muscles used in shutting it will soon thereby become sensibly affected. One should look at the same time with the left eye upon the table (the darkest possible, for example, a dark green colored one). The inclination to squinting, arising from this use of the eyes, may be easily prevented by giving the eyes timely rest after working with the microscope.*

Respecting the mental condition of the observer it may be mentioned, that — as it lies in the nature of the case — only that temperament of mind can be serviceable to investigation in which we find ourselves absolutely passionless. Says Harting,[17] "But for the exercise of the critical judgment, in microscopical observations, there is need not merely of the simple purpose, but we must find ourselves in that condition of mind which makes it possible for us to see with unclouded vision, and to judge with unprejudiced understanding. As the principal requirement thereto I name mental tranquillity during the investigation. As easy as it might seem to satisfy this requirement, experience teaches that the opposite most often prevails. In microscopical investigations this is of great importance; for these often occasion lively mental impressions which are incompatible with the desired mental rest during the observations."

Of the four above named indispensable characteristics of the microscopist, self knowledge plays the chief *role*. The microscopist must be a sceptic through and through; he must take nothing for granted; he must approach every object to be investigated with a certain mistrust. He will much more easily be trained to be a good observer if he continually seek to detect himself in a false observation, than if he goes at his work with the self-satisfied consciousness that nothing could make him see

spectacle glass so constantly strikes against the ocular, the part which lies upon the nose will be pressed upon the skin covering the bridge of the nose, in such a way as finally to produce a very troublesome pain, which may bring on headache. This discomfort may be avoided by soldering upon the bearing place of the spectacles a thin plate of gold some three millimeters wide, and 35 to 40 mm. long, which has previously been bent into the exact form of a nose saddle.

[17] Harting, *l. c.*, page 327.

* The evils here mentioned are greatly mitigated by the use of the binocular microscope, or, with monocular instruments, by employing an eye shade such as that shown in Fig. 17. R. H. W.

anything falsely or imperfectly. The microscopist must exercise self-criticism. As easy as it is to criticise others, it is very difficult for many men — perhaps for all — to lay upon themselves the strict, critical measuring rod, with which they are so ready to measure others. Egoism, plainly, rules the world. Yea, still more, not only is self-criticism necessary to the microscopist, but also love of truth for its own sake. He should not delude himself, by thinking that he has already seen this or that with exactness, or perfect correctness, when it has first darkly dawned upon his consciousness. He must constantly protect himself from accepting for positive fact, what is but probability or only possibility. How this last-named quality of the microscopist, and of all who would become such, is to be attained is not easily put down in words on paper, — in respect to that every one must go through his own self-disciplinary school, — but the inscription upon the Delphian temple, "Know thyself," should in spirit constantly hover before him.

II. The Compound Microscope.

The compound diopteric microscope[18] is an optical apparatus which, by means of a convex lens, produces a magnified image of an object, which again is viewed through a glass that still further enlarges it. The compound is distinguished from the simple microscope essentially by this fact, that by means of the latter we view the magnified object itself, while by the former we look only upon the enlarged image of the object. From this statement it must follow that the compound microscope must consist of at least two glasses, viz., of one which is brought near to the object to be magnified, and which produces the image (image producer, objective), and of a second which stands near to the observing eye, and through which the picture already produced is viewed (image viewer, ocular). The two glasses must naturally have such a mutual position in respect to each other that the image will fall exactly in the focus of the ocular. In microscopes of the present day, however, neither

[18] Compound catopteric microscope there is none — the catadiopteric is temporarily excluded from our consideration.

Zentmayer's American Student Stand.

the objective nor the ocular consists of only one lens, but the former is [most commonly] constructed of three plano-convex achromatic lenses, and the latter of two glasses of which the one (the collecting lens) is placed beneath the image and [sometimes] has a biconvex form, while the true ocular is above the image and is plano-convex with the plane side turned toward the eye. For the production of a good microscopic image it is required:

a. That these lenses shall by skilful grinding be given that curvature which corresponds to the magnification as it has been previously determined by calculation.

b. That the five lenses shall be exactly centered; that is, that their foci shall lie in one straight line in the longer axis of the microscope.

The above described optical apparatus must be mounted in a stand so constructed as to permit:

a. Objective and ocular to be placed at a definite distance from each other which remains unchanged during the observation.

b. The bringing of the object to be observed exactly in the focus of the objective.

c. The furnishing a sufficiently large quantity of light for the object, to give its image the desired degree of brightness.

I. THE MICROSCOPE STAND.

[For the purpose of introducing the forms, modifications and nomenclature of the various parts of the compound microscope as now made in this country, a few stands will be figured and described; the selection being made with a view to obtain (with the exception of plates VII and VIII) typical American forms, of moderate size and free from ostentatious display of unnecessary mechanism, and especially those which have been instrumental in bringing recent improvements into use.]

[Stands of the class represented by plates III to VI, varying in style according to the skill, ingenuity, or caprice of their makers, can be obtained from all dealers at a cost of from $25 to $30, or with a minimum outfit of objectives and accessories at $40 to $50. Stands of the class represented by plates IX,

X and XI, should cost with a minimum outfit about $50 to $75; though they are capable, by a judicious increase of expenditure, according to the needs or means of the purchaser, of being developed into instruments of a far higher and costlier grade.]

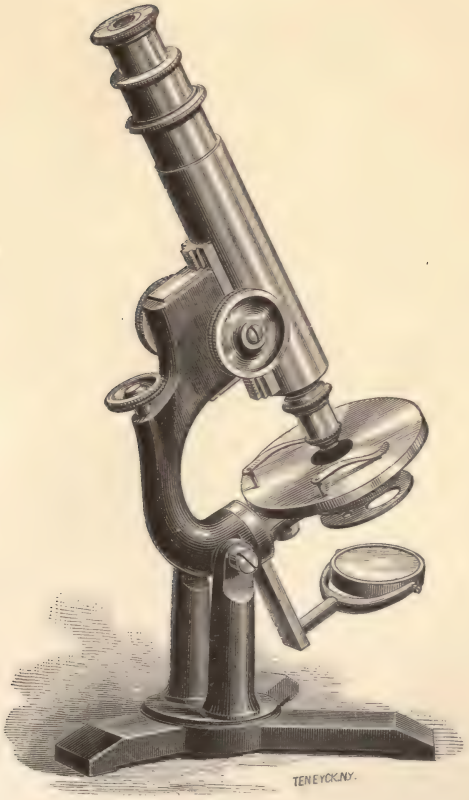
[A. The Student Microscope.]

[As a sample of the smallest, simplest, and least expensive instruments really worthy of being commended as available for scientific work, may be mentioned the "Student" microscope of Joseph Zentmayer of Philadelphia, which is represented at one-third actual size in plate III; the plate representing the parts usually known in the aggregate as the stand.]

[The foot is a support widely spread at the bottom, having three points of rest upon the table, and prolonged upward at the center into a conical pillar, which bears at its summit, by a trunnion joint capable of rest in any position from vertical to horizontal, the parts required for holding, illuminating and viewing the object.]

[The stage, occupying a central position, is a firm plate of blackened brass, nearly square in form, perforated in the optical axis of the instrument with a circular opening for the transmission of light, and designed to support the object. A glass slip, or other contrivance carrying the object, is held in position, while lying upon the stage, by two spring clips under which it is placed. The size of the central opening of the stage and the amount of light passing through it, are regulated by means of a circular revolving plate or diaphragm, let into its upper surface, and supplied with a series of apertures of various sizes, any one of which may easily be brought into use.]

[The illuminating portion is a mirror, plane on one side and concave on the other, placed below the stage, and so mounted that it can be readily turned toward any source of light. It is supported by a tail-piece or mirror-bar, a radial arm having a swinging motion around a center corresponding with the position of the object on the stage, by which motion any desired obliquity of light can be obtained with great facility, or the



The Model Microscope.

mirror can be carried above the plane of the stage for the purpose of reflecting light upon the top of opaque objects.]

[The main tube of the instrument is called the compound body. It contains an ocular, or eye-piece, slipped into its upper end and has a screw at its lower end for the reception of any desired objective. Its normal length for use in an inclined position, as shown in the plate, is partly secured by means of an inside sliding tube, known as the draw-tube, which can be pushed in for the sake of greater compactness when the stand is to be used, as is frequently necessary in laboratory work, in a vertical position. The whole compound body, carrying the essential optical parts of the microscope, slides smoothly through a fixed outside tube, so that the required distance between the magnifiers and the object can be approximately secured by a push with the hand. By using the thumb and fingers adroitly, and giving a screwing motion to the sliding tube, this adjustment can be made safely, and with sufficient precision for even moderately high powers. When greater accuracy is required it is attained by means of the fine adjustment, a sliding motion upon planed surfaces of brass just back of the compound body, this motion being controlled, with great delicacy, by means of a screw with finely cut threads acting upon an intervening lever. The milled head attached to the top of this screw appears on the extreme left of the instrument, at the top of the curved limb connecting the stage with the compound body.]

[B. The Model Microscope.]

[This microscope, made by the Bausch and Lomb Optical Co., of Rochester, N. Y., is represented in plate IV, one-third natural size. It is a rather larger instrument, of convenient form and good workmanship, having two pillars instead of one. The coarse adjustment is made by a rack and pinion movement, the large milled heads of the pinion appearing in the plate just behind the compound body; this adjustment being more convenient though not more precise, in skillful hands, than the adjustment by sliding tube. The fine adjustment screw is in the same position as before, though acting upon a clock-spring

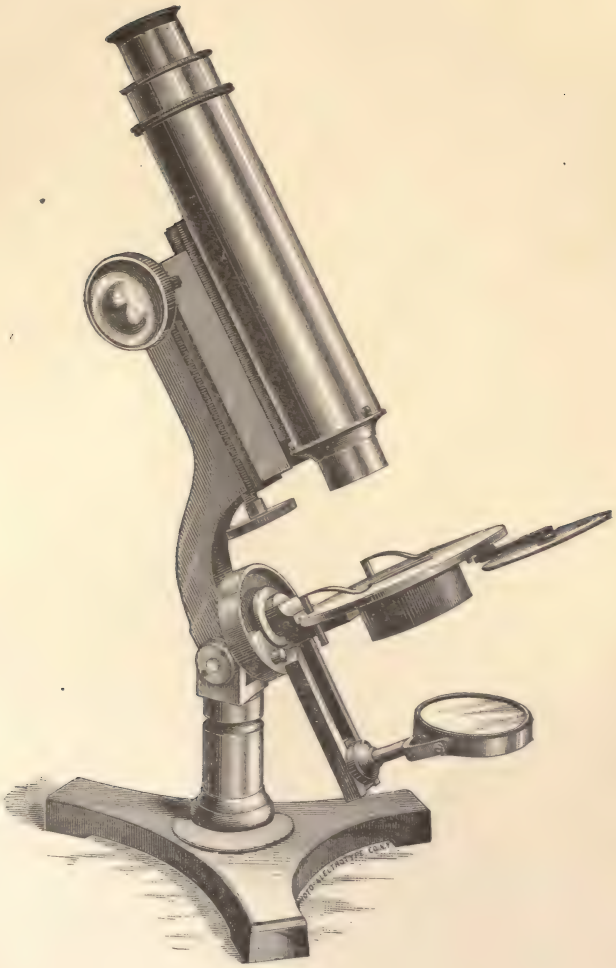
system, to be described hereafter, instead of upon a lever. The stage is round, and concentric to the optical axis of the instrument, as are all other round stages worth mentioning; and to it may be added an extra revolving plate with a movable object-carrier, by which means the adjustment of the object beneath the objective is much facilitated. The diaphragm, seen just beneath the stage, or other substage apparatus, is slipped into a substage ring provided for that purpose.]

[C. The Acme Microscope].

[Somewhat similar to the last in size and general efficiency is the new model Acme No. 4, represented in Plate V, made and sold by James W. Queen & Co., of Philadelphia. In this instrument the fine adjustment screw is removed to an exceptional location below the limb, and the pinion of the coarse adjustment is placed very high, close to the top of the limb, in order to secure the long range of adjustment required for low-power objectives. The diaphragm, being attached to a movable arm, can be swung out of position, as seen in the cut, when not in use, and a substage ring, also shown in the cut, attached in its place for the reception of an illuminating lens or other apparatus. A material advantage of this stand is the possession of a body sufficiently large for oculars of ample size; thus admitting adequate oculars of its own and permitting the frequently convenient interchange of any oculars, not exceeding that reasonable size. The diameter of the ocular is about 1¼ inch (32 mm.), which is the size recently recommended as a standard by the committee on oculars of the American Society of Microscopists, but not yet acted upon by the society. A larger and more elaborate Acme stand, No. 3, by the same manufacturers, has a body of the same size, but possesses a rotating stage and a substage, somewhat like those in Plate X; the substage being attached, however, to the same bar as the mirror, as in Plate IX.]

[D. The New Student Stand.]

[Another excellent instrument of the same grade, of the latest American type, is the New Student Stand, made by



The Acme Microscope.

Bulloch's New Student Stand.

Walter H. Bulloch of Chicago, and represented $\frac{2}{3}$ natural size in Plate VI. The peculiarity of this instrument, as compared with the preceding, is the possession of Mr. Bulloch's form of fine adjustment described hereafter. A sliding object-carrier which can be adapted to the stage is shown lying near the foot of the stand.]

[E. The Physicians' Stand.]

A very solid and serviceable instrument of this type is the Physicians' Microscope of L. Schrauer of New York, shown in Plate VIII A. The body is large, admitting an ocular of 32 mm. in diameter, and is adjustable by means of its draw-tube to any length from 16 to 25 cm. or more. The diaphragm is inserted in the stage; and a glass sliding stage is provided, in the Zentmayer style, held in position by a spring with ivory tip. Such a stage has a smooth motion and wide range, is available for use with the Maltwood finder (a photographed scale of great use for recording the exact location of mounted objects on a slide and enabling them to be promptly found when wanted again), and is unaffected by those reagents which might, in certain cases, mar a brass stage. The joint by which this stand is inclined has a set-screw for securing it in any position. The disk of the swinging mirror-bar is graduated as in all the higher class stands of this type, for the purpose of determining the obliquity of illumination or the angular aperture of objectives.]

[F. The (Continental) Student Stand.]

[While the stands heretofore and hereafter described may be considered as representing the characteristic American type, there have always been some observers who preferred the small, compact stands of the French and German model, known as the "continental" style. Some makers have accordingly adopted a model of this type. Mr. J. Grunow, of New York, one of the earliest American makers, has always been distinguished for this class of stands, and his excellent workmanship has gone far toward making them popular for medical and histological work. His Student Stand No. 2, represented by Plate VII, is a very

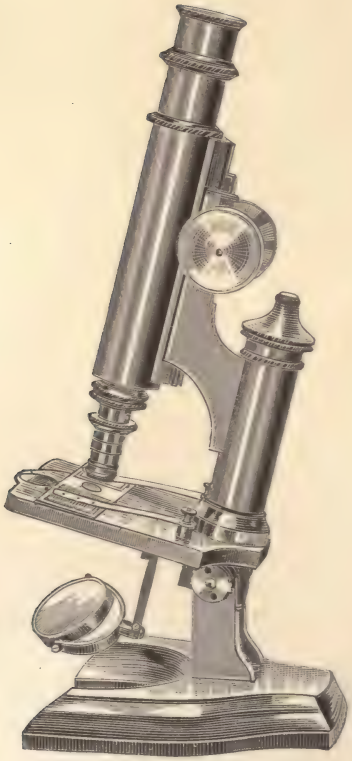
efficient instrument of this class, a solid little stand, with short body and limb, a draw-tube, a low square stage with included rotating diaphragm, and a heavy horse-shoe base.]

[G. The Illustrator's Stand.]

[Of somewhat erratic model is the microscope called the Illustrator's. It is made by T. H. McAllister of New York, and shown in Plate VIII B. It is one of the simplest and most practical of those designed to hold several objects at once. It consists of a broad circular base from the center of which rises a pillar that carries a mirror, a circular stage 25 cm. in diameter rotating concentrically about the pillar, and at the top a horizontal transverse bar with its vertical compound body. The body is focussed by sliding through a tube in the transverse bar, the motion being controlled by a pin working in a spiral slot. The stage is capable of carrying twelve slides, radially, at an equal distance from its center, which can be successively brought under the lenses by rotating the stage. The microscope is best adapted to large objects under low powers. It is adapted to certain uses in teaching, where a number of forms are to be shown in comparison with each other to a class. In research, its use is mainly limited to the rapid comparison of objects, as in the classification of unfamiliar objects, the study of adulterations, or the comparison of samples of merchandise.]

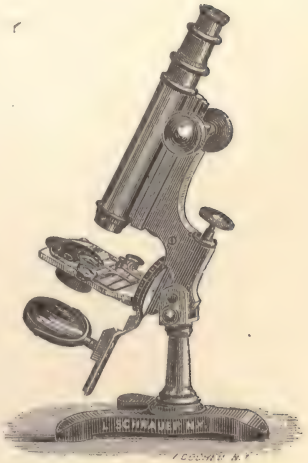
[H. The Histological Stand.]

[This stand, made by Mr. Zentmayer, and represented, with the addition of the Wenham Binocular arrangement, Fig. 16, in Plate IX, is of the same size as his Student Stand, most of the castings being identical, but is a far more efficient instrument. This superiority is due mainly to the possession of a substage, a horizontal ring or short tube, designed to support the diaphragm or other apparatus that may be required between the stage and the mirror. This substage is carefully centered around the axis of illumination between the mirror, in whatever position it may be placed, and the object on the stage; and it has a smoothly sliding vertical movement by which it may be readily located at



Grunow's (Continental) Student Stand.

PL. VIII a.

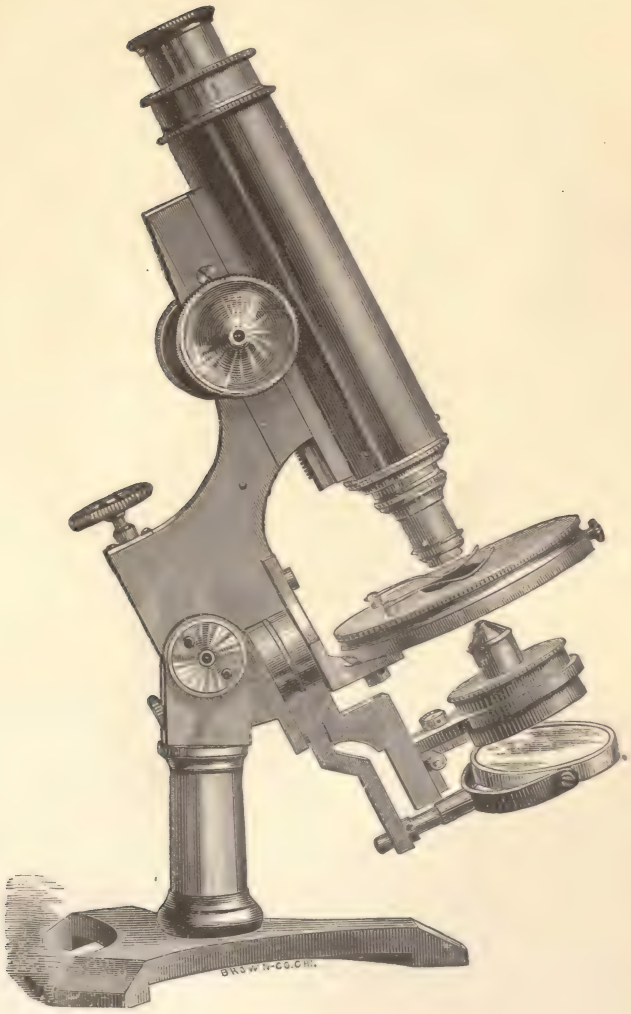


Schrauer's Physicians' Stand.

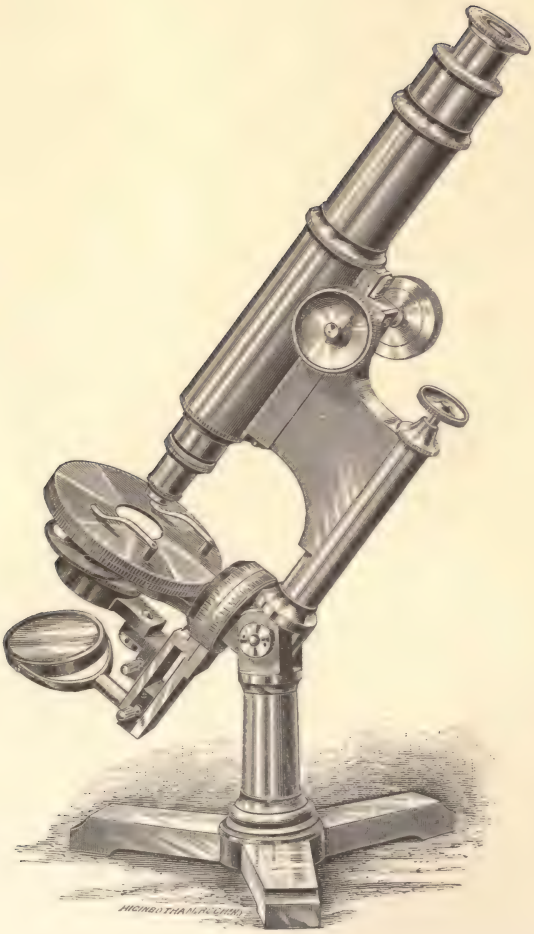
PL. VIII b.

McAlister's Illustrator's Stand.

Zentmayer's Histological Stand.



Bulloch's Biological Stand.



The Bausch and Lomb Universal Stand.

any point of that axis. This stand is made with a glass sliding stage, or with a round rotating stage, if desired. In the monocular form, the cost may be reduced by substituting for the rack and pinion coarse adjustment a sliding tube like that in Plate III. It is most commonly made monocular, as in Plate III, but it can be made binocular as figured, at an extra cost; as can also the Acme No. 3, and the Biological and Universal. It is one of the earliest instruments to which were applied several of the expedients just now termed the modern improvements of the microscope; and it presents, in combination with them, the low square stage, and the small body, of the continental style.]

[I. The Biological Stand.]

[Of larger instruments, capable of utilizing all necessary accessories, and believed by the writer to be large enough for any histological work, Mr. Bulloch's Biological Stand, represented in Plate X, was one of the first to assume substantially its present form. In this stand the tail-piece is made double, one portion carrying the substage and the other the mirror; an arrangement which is essential to the efficiency of this modern device, since the substage frequently requires to be in a position axial to the compound body, for the purpose of holding illuminating lenses or prisms, for instance, at the same time that the mirror is being used in an oblique position. The usefulness of the whole arrangement is impaired in such cases unless the different parts can be moved independently of each other.]

[J. The Universal Stand.]

[This stand, made by the Bausch and Lomb Co., is represented $\frac{1}{3}$ natural size in Plate XI. It comprises the same general features as the one last named, but by a slight increase of distance between the stage and the table sufficient space is secured to admit the use of the largest illuminating or polarizing apparatus, etc., that is usually employed on the largest stands. In fact there is scarcely any of the accessory apparatus of the highest-priced microscopes that cannot, with a few slight modi-

fications in non-essential particulars, be easily and efficiently combined with this. This stand can be obtained as shown in the cut, in a very simple and inexpensive style; but it is capable of a much higher development. It has been constructed, for the use of the writer, with the addition of lengthening mirror bar, graduated draw-tube for use in micrometry and in drawing to scale at any desired amplification, centering adjustment to stage, and graduated rotation of the same, centering substage moved vertically with rack and pinion, and graduated fine adjustment screw with index point, for use in measuring approximately the thickness of objects or cover-glasses. It is named by the makers the "Universal," from the belief that it is possessed of the working capacity of the most elaborate stands. The stage is well adapted to the use of a glass sliding stage; and a mechanical stage moved in all directions by special mechanism can be added if desired. R. H. W.]

We proceed now to the more particular consideration of the separate parts of the microscope and begin with the most important part of the optical apparatus, viz., the objective.

II. THE OBJECTIVE.

The objective consists, as we have seen on p. 15, of several achromatic double lenses joined together in a system. The objective lenses as a rule are plano-convex and are formed of a biconvex converging lens of crown glass, Fig. 1, I *a*, and a plano-concave dispersing lens of flint-glass, Fig. 1, I *b*. The under, convex side of the former corresponds exactly to the upper, concave side of the latter. The two are cemented together to form a whole by means of perfectly transparent, colorless Canada balsam. Very rarely and only in glasses made for the greatest magnification, the underside of the flint glass lens, is given a very slight concavity, Fig. 1, II, *b*, so that these in the same manner as the others become an achromatic, concavo-convex lens. Formerly

FIG. 1. I, II.

each achromatic lens was mounted by itself, and before being used several were combined according to need. This was known as a "set" of achromatic lenses. Now, at least for the larger instruments, these achromatic lenses are permanently combined in the optical manufactory and constitute the "objective system."

A. The Set of Achromatic Lenses.

Each achromatic double lens is mounted in a short brass tube, as is shown in Fig. 2, I, II. In II, *d*, is the achromatic lens with the plane side of the flint-glass lens turned downward. It is placed in the middle of the short tube in the upper part of which is cut the matrix *b*, into which the whole of the patrix of a similar short tube, I *c*, may be screwed. Each tube is designated by a successive number, and the lens of least power bears the lowest figure and that of the greatest the highest figure. In III is represented, for example, a set of lenses belonging to an old instrument of Schieck. [Such lenses may be used singly or combined into sets of two or three, the smallest lenses, when the size varies, having the highest power and being placed at the bottom of the combination. R. H. W.]

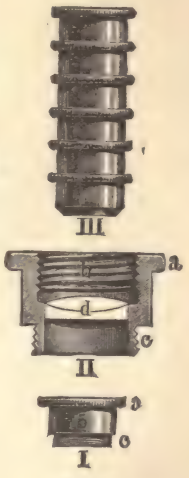


Fig. 2.

These sets of lenses were given up a long time ago, and are applied now only to the very cheapest instruments. In scientific botanical investigations we shall very seldom be in a situation where we must use them. We consider now:

B. The Objective-system.

Aside from the advantages already mentioned which follow the permanent combination of several double lenses into a system, certain essential improvements in respect to both aberrations (see pp. 5–6) may be aimed at in the proper arrangement of the lenses; for, as Lister[19] first pointed out, the special

[19] Lister in Philosophical Transactions, 1830, p. 198, ff.

aberration of double lenses may be made to compensate each other by placing them at proper distances apart. It is not possible to correct both aberrations perfectly, for the difficulties attending the construction of very small achromatic lenses with short foci is commonly very great, the grinding of the most powerful lenses being done with the help of the microscope itself. Thus the best systems of lenses are not altogether free from faults, but these faults are reduced to the lowest possible limits.

We shall just here briefly mention some general qualities upon which the value of microscopical glasses depends and which we already in the foregoing have many times designated by name.

There are two principal requirements of a good objective-system. It must give, first, a field of view of the greatest possible size and brightness, and second, an image of the greatest possible distinctness.

The quantity of light which may pass through a lens depends upon its superficial area, and the amount of light which may pass through two lenses of different sizes is proportioned to the square of their diameters. That is, a lens of n mm. in diameter will afford a field of view four times as bright as one $\frac{n}{2}$ mm. in diameter. The degree of brightness of the lens is measured by its "angle of aperture." What this is we understand by drawing as in Fig. 3, I, straight lines from two diametrically opposite points on the edge of the lens $g\,h$ to the focus B. The angle EBF in this case is the "angle of aperture." It is divided in halves by the principal axis of the lens AB.[20] In a combination of lenses the angle of aperture will not be determined by the extreme peripheral rays which will enter the front lens L from a luminous point, lying in its focus B, II, but by those which will pass through the whole combination L and L'. The angle of

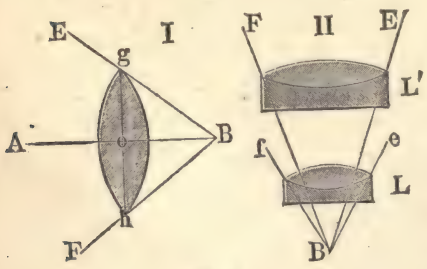


FIG. 3.

[20] The principal axis of a lens is that straight line which joins the middle point of the spherical surfaces of the lens. It also passes exactly through the middle point (o) of the lens.

aperture in the combination illustrated by Fig. 3, II, is not *eBf* but *EBF*.[21] From this it follows that an objective-system which shall give a large and bright field of vision[22] must be so constructed as to have the widest possible angle of aperture. But it has been found in practice, that there is a certain maximum, which must not be overstepped, or, in the highest magnifications, the image will be materially damaged in other respects.

The angle of aperture, as appears in the foregoing, is greatest in such lenses as have the shortest focus, and the most highly curved surfaces. But here again comes in the defect, at least if it have a *spherical* surface, which we have several times designated as spherical aberration. Let us suppose that in front of a lens, Fig. 4, in its principal axis *AB* is found a luminous

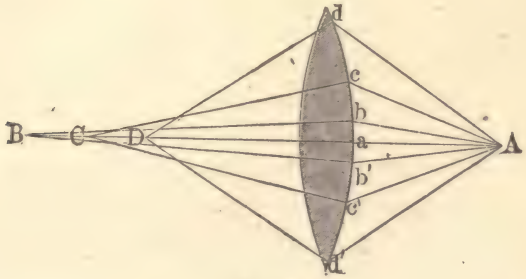


FIG. 4.

point *A*. This emits rays *Aa Ab Ac Ad* on the upper half, and *Ab' Ac' Ad'* on the lower half of the lens. The ray *Aa* falls perpendicularly upon the surface of the lens in the direction of its principal axis, and will pass through unbent, while *Ab* and *Ab'* will be bent in a definite angle, and will have their meeting point behind the lens in *B*. Likewise *Ac* and *Ac'* after being bent will be united again in *C*, and *Ad* and *Ad'* in *D*. That is to say, the farther removed the point is from the center of the lens through which a ray passes, the more it will be bent, and the shorter

[21] A very simple contrivance for measuring the angle of aperture of a microscopical objective-system is given by Dippel. (Das Mikroskop. Braunschweig 1872. Bd I. p. 86 f.).

[22] According to Dippel (*l. c.* p. 83 ff.) the resolving power of an objective-system depends upon the size of the angle of aperture. While Harting (*l. c.* p. 249 ff.) traces this back to other causes.

the distance behind the lens where they will be reunited. We get three points behind the lens where the rays which have been emitted from *A* come together again; viz., *B*, *C*, and *D*. Nay, many more, since *A* sends out not only the rays we have supposed but an infinite number of others, so we shall have an infinite number of focal points all lying between *B* and *D*. We have assumed that the rays *Ab* and *Ab'* are the nearest possible to the principal axis, and *Ad* and *Ad'* the nearest possible to the edge of the lens. This variation in the focal points we call the spherical aberration, and the distance *B* to *D*, the length of it. If now we hold a translucent screen or a piece of ground glass at the point *B*, we shall see thereon a picture of *A*, but it will be made more or less indistinct by the rays which come from the outer portions of the lens, and have crossed and been dispersed at *C D*, etc. So we must get rid of these so-called "marginal rays" *Ac Ac' Ad Ad'*, etc., by shutting them off. This can very easily be done by means of a diaphragm. To accomplish this, all that is necessary is to place an opaque disk with a circular hole in the middle of it between the lens and *A*, or the lens and *B*, so as to permit only the middle rays *Aa Ab Ab'*, etc., to pass through. This is indeed a very simple method of overcoming the spherical aberration, but it is only to a certain extent satisfactory, since naturally what such a diaphragm shuts off, reduces the angle of aperture by so much, and in consequence thereof, the field of vision will be made smaller and its brightness much diminished. It is on this account, of the greatest importance, that the spherical aberration should be reduced in some other way, viz.; by the peculiar construction of the lens itself.

If a lens be not alike convex on both sides, but if one side have less curvature than the other, or the lens be plano-convex or plano-concave, its spherical aberration will be diminished, as experience teaches, if the side of least curvature, the plane or concave side of the lens be turned towards the object (at least with microscopical objectives).[23] It has been shown that those biconvex lenses which have surfaces of unequal curvature and

[23] With telescopic lenses, whose object is very far removed, we must reverse this rule and present that side of the lens which has the greatest curvature to the object.

the least spherical aberration are those whose curvature on one side has a radius six times as long as that of the other.[24]

The spherical aberration is diminished also, by combining a biconvex lens of crown glass, and a plano-concave lens of flint glass. In a biconvex converging lens the spherical aberration is of that nature that the marginal rays come to a focus in front of the true focus of the lens. A diverging plano-concave lens has an aberration of like nature only with this important difference, that the marginal rays come to a focus behind the focus of the central rays, or the true focus of the lens. If now the two lenses be made of glass, of different refractive powers, and then combined to make a whole, and rays of light be passed through them, the relative direction of the central and marginal rays will be reversed by the action of the diverging lens. It comes about, thence, that by the united effect of the two lenses the focal point of all the rays will be brought very near together; only it must be understood that the lenses should be made with a definite curvature on their contiguous sides, which indeed we need not here more particularly specify.

Finally, there is another very effective means for materially lessening the spherical aberration, which consists in uniting three plano-convex doublets in one system. In this we follow Lister,[25] who discovered the one peculiar characteristic of the achromatic doublets, and gave formulas for their combination based on this discovery. The distance apart of the three lenses must be a very definite one. It is the art of the optician by exact and most careful experimentation to give the lenses their right position in his objective-system. The smaller the achromatics, the shorter their focal distance and the more difficult is it to accomplish this combination. It is for this reason that good objective-systems are so expensive. The great advantage which is attained by this combination of three lenses is that, notwithstanding the great magnifying power of the lenses singly, and in spite of correcting their spherical aberration, the angle of aperture is still large and the field of vision still shows great brightness.

[24] This proportion answers, however, only for those kinds of glass which have an index of refraction of 1.5.

[25] See more particularly Lister, at the place before mentioned and in Harting, *l. c.*, p. 46 f, and 138 ff.

It is a generally known fact that by uniting a biconvex crown glass lens with a plano-concave flint glass lens the chromatic aberration of the rays is almost entirely overcome. We have already stated the reasons for this on page 5. We believe that in this cursory review of the physics of the matter we shall not be expected to go into it more particularly.[26]

We will therefore only add in brief, that in refraction through a single lens, the violet rays have their focus nearest the lens, are the most converged, while the focus of the red rays is farthest removed from it. The focal points of all the other colored rays lie between, naturally, in the order in which they appear in the spectrum. An achromatic lens-combination is now so corrected that with it the focal distance for red and violet light is the same. In this way these two colors of the spectrum fall upon the same point, but not so with those which lie between. These, as is easily seen, give secondary dispersion images which appear as colored borders of different tints, commonly yellow or green. To remove these it is only necessary to make a combination of several doublets, and in fact this requirement is met in a combination such as is the present objective-system. As in these systems the spherical aberration is reduced to its lowest terms, so also the chromatic aberration is, for the most part, overcome. At the present time, if the flint-glass lens is given a very slight preponderance the result is that the system shows a very soft, and to the eye a very pleasant blue tinge, in the microscopic image. We call such a lens as that *over-corrected*. If the lens on the other hand shows a red border (by reason of a preponderance of crown glass) we say it is *under-corrected*.

Since the objective-system is capable of all the wished-for corrections, of which we have spoken, we have in it what we call an "aplanatic," that is, a lens with the least possible error, the smallest amount of both spherical and chromatic aberration.

*

* *

The Systems in Practice. Now that we have become acquainted with the optical principles of the objective-systems,

[26] See thereon for example Wüllner's Lehrbuch der Experimentalphysik, Leipzig, 1871, Bd. II, p. 216–220 —— v. Quintus-Icilius. Experimentalphysik, Hanover, 1866, p. 250ff. —— Harting, *l. c.*, p. 37–46.

we shall proceed to consider some of the different sorts of objectives.

The common system consists of three achromatic doublets of the form shown in Fig. 1, I. They are arranged as is shown in Fig. 5, *a*, *b*, *c*. The smallest, which is also the strongest magnifier, is nearest the object; the largest and weakest is removed farthest from it. By this combination there is attained on the one side a greater focal distance of the object, and on the other an objective so constructed gives a wide angle of aperture and a very bright image.

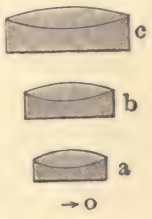


FIG. 5.

Differing from this, a very perfect system is sometimes made by combining an under lens of three parts, a plano-concave of flint glass, and two plano-convex lenses of crown glass, with a middle lens of the form of Fig. 1, I, and an upper lens constructed of a middle bi-concave of flint glass and two bi-convex lenses of crown glass.

The mounting of an objective-system is clearly represented in Fig. 6, which is a medium system of natural size from the manufactory of Seibert. The three plano-convex doublets are contained in the lower half *da*, which part is screwed into the upper *db*, the latter having no lenses. Both parts are made permanently fast to each other. At *b* the system is provided with a screw thread by which it is made fast to the microscope. In this manipulation one takes it with the thumb, index and middle fingers of the right hand, on the two edges *cc*, their milling enabling one to hold it fast. On the smooth surface *e* between the two edges the number [and maker] of the system is engraved. [Somewhat similar objective-systems are shown *in situ* at the bottom of the microscope tube, or compound body, in Plates IV and VI to XI. R. H. W.]

FIG. 6.

The use of such a system (the so-called dry lens) is very easily understood. But the use of two other systems of construction, mainly applied to the production of much higher magnifications; viz., the "immersion-system" and the "correction-system," is very much more elaborate.

The Immersion-System. In order to understand this system, it is necessary to assume that the botanical object to be viewed by the microscope lies under a thin glass plate — the cover-glass — and is surrounded by a layer of water or other fluid, glycerine, essential oils, etc. The light, which enters the optical apparatus of the microscope from the object has to pass, on its way, through one after another the several media, water, glass, air. Since the glass and the air are very different in their refractive power, and the light enters from a thicker medium, glass, into a thinner, air, a number of the rays will be so far dispersed that they will not be able to enter the objective-system, and the consequence will be that the microscopic image will be correspondingly darker; and further, a considerable reflection of the rays of light will take place from the under plane surface of the lens.

In order to remove this defect we have for a long time adopted this contrivance, with high magnifying powers, of substituting a thin film of water for the layer of air which intervenes between the cover-glass and the front lens of the objective. We owe this especially to Hartnack. Since the refractive power of the water is much nearer that of glass than is that of air, it is obvious that the interposition of the film of water will very considerably diminish the before-mentioned dispersion of the rays of light, and will cause therefore, many more to enter the objective and will give to the microscopic image a considerably greater brilliancy. The result of this arrangement is essentially the same as if the angle of aperture of the objective were considerably increased. By this means the reflection of the rays from the under surface of the lens and also from the upper surface of the cover-glass is altogether obviated. Such a system, whose lowest lens is immersed in water is called a "water-system" or "immersion-system." This objective is prepared for use in the following way. The objective is turned bottom side up, and a drop of distilled water from a flask, by means of a glass rod or hair pencil, is placed upon the lens. Here it will round itself up into a little hemisphere. Now reverse the objective and the drop will remain in place by the power of adhesion. The objective should now be screwed into

the microscope tube, and the tube pushed down by hand or by the rack and pinion till it comes near the cover-glass upon the object which lies upon the stage. Now if one breathes a little upon the cover-glass and then carefully brings the drop down till it touches it, it will easily unite with the surface of the glass and form the desired film of water between the glass and lens. Then, by means of the fine adjustment screw, the object can be exactly focussed. Particular care should be taken that the drop of water used does not contain even the smallest bubble of air, else the microscopic image will be ruined. After use, the drop of water which adheres to the objective should be carefully wiped off with a piece of soft old linen cloth which has been washed in distilled water.

[It is sometimes, moreover, convenient to plunge the objective directly into the medium in which objects are situated, for the purpose of examining them without preparation or selection, and under strictly natural conditions. When the medium is not corrosive, ordinary "immersion" objectives may be used in this manner, provided they have sufficient screw-collar movement to make the necessary corrections; and objectives specially corrected for this use have been constructed by Tolles and others. Such lenses thus used, though not inapplicable to certain botanical researches, have been heretofore mostly employed in zoology and pathology. By a modification of this plan, however, dry lenses of lower powers may acquire a new value to the botanist. By surrounding the objective with a brass cylinder open above and closed tightly with a thin cover-glass below, it may be plunged into water or various solutions, with impunity, and a clear and satisfactory view may be obtained of objects in the fluid, or lying at its bottom, in saucers, dissecting troughs, or other suitable vessels. Of course the vessels, or their bottoms, must be of glass if transmitted light be required. By this method, not only the unavoidable tremor of the upper surface of the liquid, which renders study of objects below by usual methods difficult and quite unsatisfactory, is rendered harmless; but the object may be freely manipulated with needles, scissors, or dipping tubes, under objectives of from two inches (51 mm.) to a low-angled $\frac{1}{2}$ or $\frac{1}{4}$, without interrupting the

view. Small objects, in small quantities, may be thus examined under the higher powers in watch-glasses. For larger quantities and lower powers, nothing is more convenient than the little glass dishes occasionally sold as individual butter plates, or the glass jars sold as seed and drink cups for bird cages. When the quantity of material is unlimited, and especially when manipulation is required, nothing is more convenient than plain glass preserve dishes one inch (25 mm.) deep, and four inches (10 cm.) wide. For dissecting purposes with reflected light exclusively, china dishes, or the hard rubber dissecting troughs sold by microscope dealers, may be used; the bottoms being lined with thin sheets of cork if it be desired to fasten down the objects with pins. A most convenient apparatus for this quasi-immersion use of dry lenses is the "objective-protector," Fig. 7; shown in section and *in situ* upon the objective in Fig. 8. It was proposed by Mr. R. E. Dudgeon of London, and is made by J. H. McAllister of New York. R. H. W.]

FIG. 7.

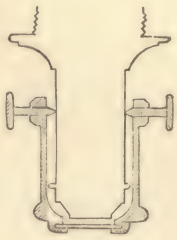


FIG. 8.

The Correction-System. We will now suppose that an object Fig. 9, *p*, lies on the microscope, under the cover-glass DD (a highly magnified section in the illustration) through which we send rays of light *pv*, *pa*, *pc*, *pe*, . . . *pa'*, *pc'*, *pe'*, from the illuminating mirror, to the objective. The rays impinge upon the under surface of the cover-glass, and those which fall upon it obliquely do not proceed in the same direction as heretofore, but, at the surface between the water and the glass, are refracted and within the glass take the direction *aA*, *cC*, *eE*, ... *a'A'*, *c'C'*, *e'E'*. Further, in passing through the upper surface of the cover-glass into the air or into the water, as the case may

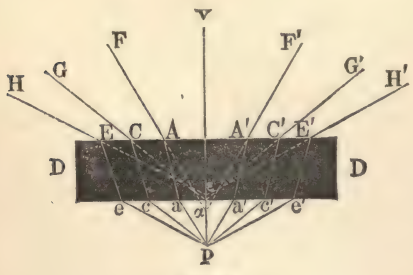


FIG. 9.

be, in dry or immersion objectives, they are again refracted in the direction AF, CG, EH, . . . $A'F'$, $C'G'$, $E'H'$. The rays of light are more widely dispersed on emerging in proportion to the acuteness of the angle at which they enter the cover-glass from p. If we now construct the uniting points of the twice refracted rays we shall have a series α—ε along the line pv, one point above another, each of which represents the image of the preparation p, as a luminous point. The distance α—ε will be less or greater in proportion to the thickness of the cover-glass DD. There results from this exactly the same phenomenon that we have learned to know as spherical aberration. The use of the cover-glass, therefore, will cause a certain indistinctness of the microscopic image similar to that caused by the use of an objective which has not been corrected for spherical aberration.[27]

We have already learned that the defect of spherical aberration can be corrected by placing the three objective lenses in a certain right relative position to each other. It is also evident, that the optician can likewise eliminate the damaging influence of the cover-glass, if the same thickness of cover-glass were always used, and he had taken into account this particular thickness in correcting the objective for spherical aberration. This is now almost always done and the objectives for medium magnifications are commonly so corrected that they give the clearest possible image with the use of a cover-glass .1 to .2 mm. thick.

For high power objectives, chiefly for strong immersion lenses, with which the influence of the cover-glass is most damaging to the clearness of the image, we have followed Ross[28] in hitting upon a contrivance which almost entirely eliminates this bad influence, viz., a device for changing at will the relative distance of the lenses, and thus obtain an objective which is aplanatic for every thickness of cover-glass. We name this the "adjustable lens," the "correction system," the "system with correction for thickness of cover-glass."

[27] More particularly H. v. Mohl, *l. c.*, p. 157*ff.*—Harting, *l. c.*, p. 146–149.

[28] Ross discovered this influence of the cover-glass in 1837 and sought at once to obviate it by the screw correction in the system (Harting, *l. c.*, p. 747). Already before this (1829) Amici had made the same discovery, but constructed however, no correction system, but added to his microscopes several equivalent systems which were intended for different thicknesses of cover-glass (Harting, *l. c.*, pp. 148, 720).

Ross so constructed his objectives that the correction was made, by changing at will, by means of a screw contrivance, the distance between the upper lenses which were fastened together as a whole, and the lower lens. Afterwards Hartnack modified this so that the two lower lenses which were made fast together were made movable towards the upper which was fixed in the microscope tube. Finally, several, especially German makers, have modified the system of Hartnack so that the two under lenses are made fast together and to the microscope tube, and the upper lens is mounted in an inner movable sheath so as to be adjusted to the other two. The last two methods of construction do not differ at all in regard to the results produced, and the difference between Ross' and Hartnack's system of correction is of a subordinate nature. In Fig. 10 is represented a longitudinal section through a Hartnack objective which shows the characteristics of the adjustment. By means of the screw thread *b* the inner cylinder *i* which bears the upper lens 3 is made fast to the microscope tube. The two under lenses 1 and 2 are screwed fast to the outer cylinder *a* into which the inner shell *i* exactly fits. At *c* is a ring which by means of a screw thread can be moved up and down on *i*. This ring bears in an inner groove *v* the cylinder *a*. Now if the ring be screwed up, the tube *a* with the lenses 1 and 2 will be carried up and not at the same time turned around the axis of the objective with the rotary motion of the ring.

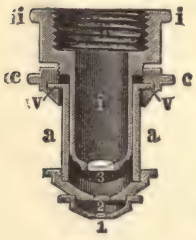


FIG. 10.

In reference to manipulating the correction in microscopical work the following may be said. Fig. 11 represents the immersion No. VII of Seibert, natural size. The objective has a screw collar *c* corresponding to *c* in Fig. 10, which on its upper smooth part is garduated into ten divisions (1, 2, 3, etc.) which graduations play by a mark at *e*. By a full turn of *c* the upper lens is removed from or brought nearer to the lower lenses, as the case may be, by the distance of a screw thread.

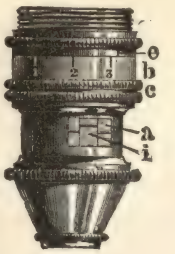


FIG. 11.

The interval between the graduations corresponds to .1 of this change of distance. Now to enable one to control this movement of the upper lens up and down from a certain medium position the device *a i* has been contrived. A small slit is cut through the outer cylinder *a* which allows the inner cylinder *i* to be seen through it. Both *a* and *i* are provided with a mark. If they stand at the same height (as they do not in the illustration) then the upper lens is in a normal position to the under. If the middle line *i* is above the other *a* the upper lens is moved farther away from the lower, and the reverse. After the lenses have been put in their normal position by bringing the 0 of the ring graduations to the mark *e*, the objective should be put on to the microscope, focus the object and then turn the ring experimentally back and forth till the image becomes very sharp and distinct. When this is done a note should be made on the slide, of the direction and distance which the ring has been moved.

III. THE OCULAR.

We have already seen on p. 15 that the ocular consists of two glasses, the upper one being placed near the eye and is the essential image-viewer, and in the narrow sense the ocular-glass, while the under one called a "collecting lens," is only in a limited sense a part of the ocular and might with much greater propriety be considered a part of the objective. But since they are always united to make this part of the microscope it has always been customary to designate the two glasses together as the ocular.

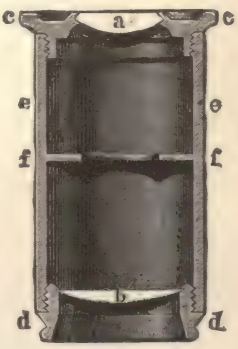


FIG. 12.

Fig. 12, which represents a somewhat conventional longitudinal section of a Hartnack ocular, will enable us to understand the arrangement of oculars. In a cylindrical brass shell, which exactly fits into the tube of the microscope, there are screwed two end pieces, one *c* above, and the other *d* below, of which the one

carries the ocular lens at *a* and the other the collecting lens at *b*. Inside of *e* at about equal distance from *a* and *b* is placed the diaphragm *f*, intended to cut off the marginal rays coming through *b*, which are so deleterious to the beauty of the image. Both lenses have a plano-convex form, and the convex side of both is downward. This arrangement of the lenses with their curved sides down, essentially influences the size of the field of view and the sharpness of the image. A reversal of them would materially diminish the field as well as the sharpness and flatness of the image.

The ocular is set in the top of the microscope-tube. The ocular lenses should be exactly centered with the objective lenses, that is, so made that a straight line drawn through the middle

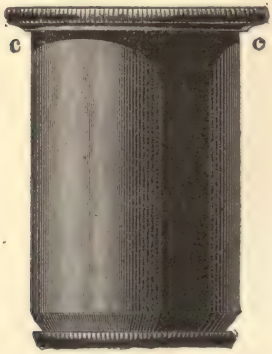


FIG. 13.

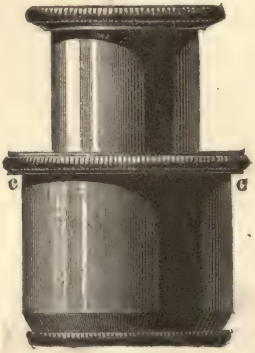


FIG. 14.

point of the objective lenses shall also, on being prolonged, pass through the middle point of the ocular lenses. This presupposes exact workmanship. Oculars have the form of a simple cylinder Fig. 13, or in later times that represented in Fig. 14. The first is found in the microscopes of Hartnack, Merz and Nachet, and the second in those of Gundlach and Seibert [and in those of most English and American makers. R. H. W.] That part of the ocular which slips into the microscope tube should exactly and easily fit into it. In putting the ocular into the microscope-tube it should always be allowed to sink down to *cc* Figs. 13, 14, in the tube. The distance of the ocular from

the objective is a definite one and should not be changed at will, save in those cases to be described farther on in treating "microscope-tubes."

For the ocular is placed at such a distance from the objective that the image produced by it would appear above the collecting lens. In other words the collecting lens is below the point where the cone of refracted rays from the objective would meet.

If we suppose a cone of refracted rays of a magnified object, from the objective, to be represented by aA, bB, cC, dD, eE, $b'B'$, $c'C'$, $d'D'$, $e'E'$, Fig. 15, it would present itself to us in

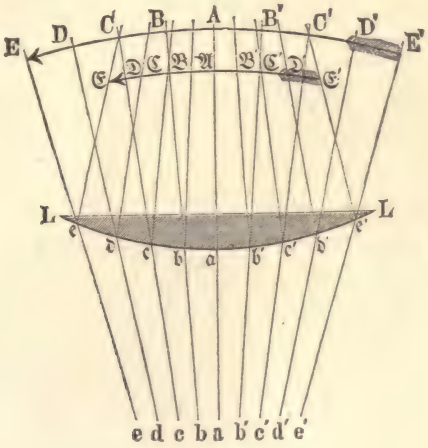


FIG. 15.

the extended form represented by EE'. Now we shove down beneath this image the collecting or "field lens," LL, corresponding to b, Fig. 12, and the rays which fall upon it at $\mathfrak{b}$, $\mathfrak{c}$, $\mathfrak{a}$, $\mathfrak{e}$, $\mathfrak{b}'$, $\mathfrak{c}'$, $\mathfrak{a}'$, $\mathfrak{e}'$, will be refracted inward toward the axis aA, and take the direction $\mathfrak{b}\mathfrak{B}$, $\mathfrak{c}\mathfrak{C}$, $\mathfrak{a}\mathfrak{D}$, $\mathfrak{e}\mathfrak{E}$, $\mathfrak{b}'\mathfrak{B}'$, $\mathfrak{c}'\mathfrak{C}'$, $\mathfrak{a}'\mathfrak{D}'$, $\mathfrak{e}'\mathfrak{E}'$, the diverging bundle of rays changed to a converging, and the image thus modified by the field lens will fall at $\mathfrak{E}$ $\mathfrak{E}'$. The collecting lens has really diminished the image. But this loss of magnification is in various ways an advantage, for now more of the image can be seen than when the rays are diverging. It is also evident that the interposition of the field lens increases

the brightness of the image by concentrating the given number of rays upon a smaller surface. In like manner the distortion of the image by the unequal magnification of the central and marginal portion is obviated. To this end also the diaphragm mentioned above affords no small help by cutting off most of the distorting marginal rays.

The effect of the ocular glass *a* Fig. 12, is that of a simple magnifying glass, by which the picture at E E′ is enlarged. It is not necessary to mention that the ocular lens should be so placed that the image E E′ will be exactly in its focus, or that combining the ocular and field glass with the objective will materially assist in correcting its spherical and chromatic aberration. They can be adjusted by somewhat over correcting the aberrations of the objective and somewhat under correcting those of the oculars. The oculars of most microscopes have the arrangement just now described. It is called the Campanian or Huygenian, ocular (negative ocular). This is at the present time sometimes so altered that the field lens is made of a concavo-convex flint-glass and a biconvex crown-glass cemented together with Canada balsam, thus making a biconvex achromatic doublet. This modification of the Huygenian ocular was first made by Kellner and was called the orthoscopic ocular. The aplanatic ocular of Plössl is much the same thing, made of two achromatic plano-convex lenses, and are combined for the most part as shown in Fig. 12.

A very useful variation is found in the Ramsden or positive ocular. The lenses have the same form as in Fig. 12, but the field lens is permanently reversed, turned with its plane side toward the objective, while its distance from the ocular lens is much less than in the Huygenian oculars. For common observations the positive ocular is seldom used. It is mainly useful in fine microscopical measurements, but is not absolutely necessary even for these.

[IV THE BINOCULAR OCULAR.]

[For the sake of the advantages of stereoscopic vision, and of the comfort secured by using both eyes instead of one, the

pencil of rays above the objective, or above an erector inserted within the draw-tube, is sometimes divided into two portions, one of which is transmitted to each eye. When the apparatus is permanently attached to a modification of the compound body, it is termed a binocular microscope, and when mounted separately and capable of removal like simple oculars, it is called a binocular ocular, or eye-piece. Among those who, when the stereoscope was an interesting novelty, undertook to apply its principles to the microscope, the first to succeed was Prof. J. L. Riddell of New Orleans, La., who is therefore justly credited with the invention of the binocular microscope. He bisected the pencil of rays above the objective by a pair of rectangular prisms which turned the parted halves of the pencil, by internal reflection, horizontally across each other to a distance apart equal to the distance from each other of the pupils of the eyes, at which points they were again reflected directly upwards by a second pair of prisms to the two oculars. Shortly afterwards, Nachet of Paris slightly modified and improved this plan, dividing and crossing the rays by internal reflection from the opposite sides of a single equilateral triangular prism, the rays passing thence to a pair of prisms below the oculars in a direction not horizontal but inclined upward. Not long afterwards, the late R. B. Tolles of Boston, still further improved this apparatus by transferring the whole system of prisms to a position just below the oculars and above an erector attached to the lower end of the apparatus, the whole being removable together, and leaving the tube ready for the reception of any simple ocular. Notwithstanding its excellent workmanship, good definition, fine stereoscopic effect, ease of removal, and applicability to all powers however high, this ocular never came into use, less perhaps on account of its considerable cost, than because of the small, high power oculars and parallel tubes required in its construction. Oculars of high power are tiresome to the eyes at best, and especially when the light has also passed through the numerous refracting and reflecting media composing an erector and set of reflecting prisms; and to preserve the parallelism of the axis of the eyes as required for distant vision, when looking intently at an object known to be

near, is a continual strain, since the eyes under such circumstances tend instinctively to turn their axes in a converging direction towards the object of vision. At least, these seem to be the causes of failure to the writer, who has endeavored perseveringly at various times since the introduction of this binocular to overcome the difficulties of its use, but always with such loss of comfort as to lead to an abandonment of the attempt.]

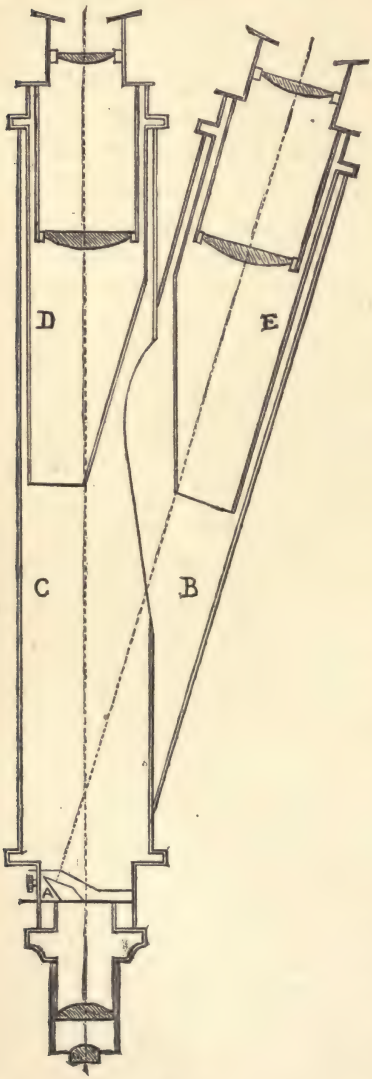


FIG. 16.

[Lately, Mr. Stephenson of London has devised a binocular in which the pencil of light is bisected by a pair of small prisms inserted very close to the objective, the image being erected and reflected obliquely forward by a mirror or prism placed in the tube above. This arrangement requires the stage to be permanently fixed in a horizontal position, the diverging tubes thus taking an inclined position favorable for every use. It is not convertible to a monocular. Being an erecting binocular it is especially adapted to use as a dissecting or preparing microscope.]

[Meanwhile, Mr. F. H. Wenham also of London had abandoned the idea of a symmetrical division of the rays, and introduced a little prism into the pencil just above the objective, which should reflect the right half of the pencil obliquely

across the left half to the left eye, while the left half passed without interruption to the right eye, as shown in section, Fig. 16, where A is the "Wenham" prism which reflects half the light through the oblique body B, and D, E are two draw-tubes by slightly raising or lowering which, the distance apart of the two eye lenses may be adapted to the eyes of various observers. Such a binocular is shown, complete, in Plate VII. This arrangement gives one field of vision with definition absolutely unimpaired, an advantage as yet gained by no other binocular. It is also interchangeable while in use from the binocular to the monocular effect, by simply slipping the prism into or out of the pencil of light. These advantages proved greatly to outweigh its somewhat cumbersome mounting which is not removable from the stand, the unequal light and definition of the two fields, and its practical limitation to medium powers and apertures; and it immediately became and has thus far remained the binocular of England and this country. It is best adapted to powers of from one to two inches (25 to 51 mm.) up to one-half or one-fourth inch (13 to 6 mm.). With low powers of ample aperture the capacity of the objective is sensibly reduced by the brass mounting of the prism, which serves as a diaphragm to cut down this aperture, and with powers of from one-half inch (13 mm.) upward, the angle of aperture of the objective used should be moderate, and the illumination should be arranged to light both fields freely without flooding the object with two much light.]

[Nachet soon changed his binocular so as to embody some of the advantages of the Wenham form, likewise incorporated into the stand itself, allowing one portion of the pencil to pass without intentional alteration through glass with parallel surfaces directly to one ocular, while the other portion was diverted twice by internal reflection in two prisms, and then directed obliquely to the other ocular. This form was well adapted to the small stands of continental model, and is still in use.]

[Just now another binocular, in the form of a removable ocular, is coming into use, devised by Prof. Abbe and manufactured by Zeiss of Jena. In arrangement of the parts and direction of the rays it resembles the last mentioned form, but

instead of a division of the light into lateral halves a part of the light from the whole pencil is transmitted to one eye, and the other part reflected to the other. By stops over the eye lenses, cutting off the opposite lateral portions of each, the fields can be so differentiated as to produce a stereoscopic effect when desired.]

[Of the numerous ingenious and plausible binoculars invented, the above mentioned are all which have come into actual use sufficiently to demand notice. It is evident that the binocular has been received with more favor here and in England than on the continent, for the reason that it is better adapted to the work of the one than to that of the other. It is most applicable to medium powers and low angles, and is most valued by those who use such powers for general natural history work, where stereoscopic effect is available and serviceable. On the other hand, it is least valued or used, if at all, by histologists, medical microscopists, diatomists, and other classes who use mostly the higher powers and larger angles, that render its use less satisfactory if not inexpedient. — R. H. W.]

[V. THE EYE SHADE.]

[When the monocular instrument is used, the fatigue of the observer's eyes is greatly lessened by habitually keeping the unemployed eye open, and protecting it by a black eye-shade, placed before it. The greatest comfort is attained when light is arrested by a central stop of limited size, which does not wholly darken the eye, but only prevents the formation in it of an image of the objects on the table. This prevents the confusing effect of an image in the unused eye, and the fatiguing effort required to keep the observer's attention confined exclusively to the microscopic image in the other; but still avoids tiresome contrast between the two eyes by allowing the entrance of much diffuse light, and permits, without abrupt transfer from darkness to light, the frequent changes required from rest over the shade to inspection of books or of other objects upon the well-lighted table. For this reason, probably, and for their own clumsiness, the large shades or hoods first used were soon aban-

doned. Lately, some very neat and useful shades have been used; but being attached to the top of the ocular, they could be used only on oculars of exactly similar size and form, and were invariably removed and required to be replaced with every change of ocular. For these reasons the writer caused to be constructed a new form represented in Fig. 17, which springs upon the compound body just below the ocular, and without regard to the style of ocular or finish of tube, is securely held at

FIG. 17.

a convenient distance from the face, is not affected by change of oculars, and can be instantly transferred to any microscope body of suitable size, or reversed to shade the other eye. Being made of hard rubber it is of proper color and light weight, is little inclined to scratch the brass work with which it comes in contact, and is sufficiently elastic to be placed without alteration upon tubes varying 6 mm. in diameter. It is made by the Bausch and Lomb Optical Co., of Rochester, of any size desired, and is cheap as well as efficient. The writer's experience leads him to believe that some such contrivance should always remain fixed upon any minocular microscope while it stands upon the table ready for use, even occasional glances into the tube being more tiresome without this accessory than with it.—R. H. W.]

VI. THE MAGNIFYING POWER OF THE MODERN MICROSCOPE.

The optical powers of a microscope depend on several different factors. A microscope which will satisfy modern requirements must give a large, flat and bright field of vision, in which the magnification of the margin is essentially the same as that of the middle. The image produced must be perfectly distinct on the edges. The fine structural relations of the object must

be sharply brought out, and the magnifying power of the instrument should not be too small. The size and brightness of the field of vision depend chiefly upon the angle of aperture of the objective, and on the proper position of the field lens of the ocular, while the sharpness and colorlessness of the image are dependent upon the correction of the spherical and chromatic aberration.

Under the designation "resolving power," we understand the capacity of the objective to bring to view the fine structural relations of the object; the more and finer these relations which an objective will discover, the greater is its resolving power.

Independent of this quality is the magnifying power of the microscope, that is, the capacity to produce an image of an object which exceeds in extension many-fold the object itself. In microscopical magnification we speak only of linear enlargement. It is expressed in terms of one dimension of space. There are various contrivances and methods to determine the magnifications of a microscope; they are all, however, grounded upon the use of a very fine measuring scale (*e. g.*, a millimeter divided into 100 parts), which is made on a glass slip and viewed under the microscope to determine the distances apart of two or more division marks of the millimeter, so as to find thence by simple division the amount of the magnification. Latterly the opticians very exactly determine the magnification of the various combinations of their lenses and furnish a table of the same with their microscopes, so the microscopist is now seldom required to determine for himself the magnification of an object; for this reason he is referred to the more comprehensive works of other authors.[29]

The magnification is produced in great part by the objective and in a less degree by the ocular. Each objective will show different magnifications by the use of oculars of different powers, since the image produced by the object will be more powerfully magnified by oculars of greater curvature than by those of less.

[29] See Harting, *l. c.*, p. 131, p. 244*ff.*; Harting, Recherches Micrometriques—H. v. Mohl, *l. c.*, p. 215*ff.*—Jacquin in Zeitsch. *f.* Physik & Mathematik. 1828 [IV], p. 1.—Ettinghausen *ibid* 1829 (V), p. 316*ff.*—Pohl in Berichte d. k. k. Acad. d. Wiss. Wien, XI, p. 504*ff.*—Dippel, *l. c.*, p. 92–100, etc.

In order to show in what relation these different glasses sometimes stand, we will take an example from the magnifications which the Hartnack oculars 1–6 give in combination with his objectives No. 2, 4, 5, 7, 10.

	OCULARS.					
OBJECTIVES.	1	2	3	4	5	6
2	25	30	45			
4	60	70	90	140		
5	100	125	160	240		
7	200	240	300	450	600	750
10	520	600	750	1100	1500	1800

It is not a matter of indifference in what manner the oculars are combined with the objectives. The principal burden should always be laid upon the objective. For the production of a given magnification, there should be combined the strongest possible objective with the weakest possible ocular. Resolving power, the delineation of the details of the image, is alone an attribute of the objective. The ocular does indeed more or less enlarge the image produced by the objective, and indeed, naturally at the cost of its brightness, but it will have only the least possible influence upon its improvement, on the resolution of the finer structural relations of the image. Supposing we desired to produce a magnification of, say, 240 diameters, in accordance with the above quoted table, we should not combine ocular 4 with objective 5, but objective 7 with ocular 2. Or if we desired a magnification of 600 diameters, we should not take objective 7 and ocular 5, but objective 10 and ocular 2. Or if we wanted 750 diameters, we should not put objective 7 with ocular 6, but objective 10 with ocular 3.

The amount of linear magnification attainable to-day with a good microscope is a very considerable one, especially is that of the immersion-systems abreast of what formerly could be produced with dry lenses, and was called a very prodigious one. In order to give an idea of what this really is, we will instance the *objective* magnifications of instruments from some of our best

optical manufactories. In each case, only the linear magnification will be given which would be produced by the use of the weakest ocular which the firm furnishes. An asterisk (*) indicates that the magnification is produced by an immersion-system, the rest are by dry lenses.

TABLE OF MAGNIFICATIONS.

MAKERS.	OCULARS.	OBJECTIVES AND MAGNIFICATIONS.
Nachet.	No. 1.	No. 0 1 2 3 4 5 6 7 8 9 10 11 12 30, 89, 180, 260, 300, 350, 460,* 580,* 775,* 900,* 1150,* 1320, 1700.*
Hartnack.	No. 1.	No. 1 2 3 4 5 6 7 8 9 10 11 12 15, 25, 50, 60, 100, 150, 200, 250, 350, 410,* 520,* 600,* 710,* 13 14 15 16 17 18 820,* 930,* 1040,* 1200,* 1400,* 1560.* No. 9 is both dry and immersion.
Seibert.	No. 0.	No. 00 0 I II III IV V VI VII VIII IX X 10, 18, 30, 45, 66, 100, 200, 305, 460,* 650,* 950,* 1450.*
Schieck.	No. 0.	No. 1 2 3 4 5 6 7 8 9 10 11 12 20, 40, 70, 90, 150, 200, 275, 400, 450, 500,* 600,* 750,* 13 14 15 16 850,* 930,* 1100,* 1400.*
Zeiss.	No. 1.	No. a aa A AA B BB C CC D DD E 5, 18, 45, 70, 110, 180, 220,* 240, 380, 400,* 680.*
Winkel.	No. 1.	No. 1 2 3 4 5 6 7 8 9 10 11 25, 54, 74, 102, 184, 222, 275, 366, 458, 500, 584.

In the following table is presented the strongest *ocular* magnification of the above named six firms.

MAKER.	OBJECTIVE SYSTEM.	OCULAR.	MAGNIFICATION.
Schieck.	System 15.	No. 5.	6500* Diameters.
Hartnack.	" 18.	No. 6.	5400* "
Nachet.	" 12.	No. 4.	4500* "
Seibert.	" X.	No. III.	4400* "
Zeiss.	Imm. " 3.	No. 5.	2300* "
Winkel.	" 11.	No. 6.	1600 "

[In America and England, objectives are universally named from their equivalent focal lengths in inches; a one-inch objective, for instance, being a lens system whose amplifying power is the same as that of the simple lens of the specified focal distance. This nominal focal distance is usually much greater than that called the working focal distance, between the object and the nearest portion of the combination of lenses. Oculars are similarly named by several makers. It follows from this most convenient system of nomenclature, that the amplification is approximately made known by the name. Thus assuming, according to usage, the round number 10 inches (25 cm.) to be the distance of most distinct unaided vision, a one inch (25 mm.) lens would magnify ten times, a two inch (51 mm.) five times, a one-half inch (13 mm.) twenty times, a one-fifth inch, (5 mm.) fifty times, etc. Furthermore, the combined amplification of objective and ocular, as used in the compound microscope at a distance of ten inches (25 cm.) from each other, will be represented by the multiple of their individual powers; a one-fourth objective, for instance (× 40) with a one-inch ocular (× 10) giving a resultant power of 400. The following table shows the theoretical powers, thus calculated, of a few combinations, other powers being in the same proportion:

OCULARS.			OBJECTIVES.									
FOCAL LENGTH.		POWER.	FOCUS. mm. / In.	5	3	1	$\frac{1}{2}$	$\frac{1}{5}$	$\frac{1}{10}$	$\frac{1}{15}$	$\frac{1}{20}$	$\frac{1}{30}$	$\frac{1}{50}$
				127	76	25	13	5	2.5	1.7	1.3	0.8	0.5
			POWER.	2	4	10	20	50	100	150	200	300	500
In.	mm.		TOTAL AMPLIFICATIONS.										
2	51	5		10	20	50	100	250	500	750	1000	1500	2500
1	25	10		20	40	100	200	500	1000	1500	2000	3000	5000
½	13	20		40	80	200	400	1000	2000	3000	4000	6000	10000
¼	6	40		80	160	400	800	2000	4000	6000	8000	12000	20000

[Such tables have been used for an indefinite period as a means of assisting the memory in respect to the powers employed. They have not been used with the understanding that

they were precisely accurate as applied in practice, but with the distinct explanation that they were only approximately correct, since it would be required, to make them literally correct, to locate the ocular and objective by means of certain optical planes which obviously do not coincide with any recognizable part of the mounting, and which cannot be determined, by any known method, with sufficient facility for popular use. It has, therefore, been somewhat customary to approximate, by taking an eight and one-half inch (21 cm.) length of tube, assuming that the rest of the ten inches (25 cm.) will be more or less evenly supplied by the mounting of the objective and ocular. The position of the planes certainly vary much in different lens systems of equal power, but they are stated to be usually, near the diaphragm of the ocular, but more or less behind the back lens of the objective. This explains the over-estimate of amplifications, especially in the lower powers, obtained by computation. It also shows that exact accuracy in such estimates is scarcely to be expected under any plan that may be adopted, and that to attain even average accuracy, it would be necessary either to lengthen the tube to an inconvenient extent, or else to habitually underrate, by general agreement, the powers of either the oculars or the objectives.]

[The following table represents the magnifiying powers of the Bausch and Lomb objectives and oculars in their various combinations, determined by actual measurement with a microscope tube-length of eight and one-half inches (21 cm.) in addition to the objective and ocular.]

OCULARS.		OBJECTIVES.													
		4	3	2	1	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{4}{10}$	$\frac{1}{4}$	$\frac{1}{5}$	$\frac{1}{6}$	$\frac{1}{8}$	$\frac{1}{10}$	$\frac{1}{12}$	$\frac{1}{16}$
A	Amplifications.	12	18	25	46	50	92	130	210	275	325	400	550	650	800
B		15	23	30	54	70	110	160	250	325	390	490	650	775	980
C		23	30	45	80	90	165	240	375	485	580	750	970	1160	1500
D		30	45	60	108	140	220	320	500	650	780	980	1300	1550	1960

[By including in this table, objectives up to one-fiftieth, and oculars up to one-fourth or one-eighth, all of which are made, the resultant powers would extend well up into the tens of thousands; such powers, however, are but little used or esteemed. These oculars do not correspond to even fractions of an inch, and are here designated by letters, after the early English style which is not yet wholly obsolete; but the makers intend hereafter to conform to the numerical plan.]

[As the nominal focal lengths of objectives, while liable to moderate errors and discrepancies, indicate the amplifying powers sufficiently well for most purposes, it is only necessary in stating the powers made by the American manufacturers, to tabulate the equivalent focal lengths of their objectives. As to oculars, the lowest is usually a one and one-half or two inch (38 to 51 mm.); the one inch (25 mm.) is much used and is said to be the power employed by prominent makers in correcting their objectives, and those of from one-half inch (13 mm.) upwards are used for such special purposes as micrometry, or counting fine lines.]

[The amplifications with two inch (5 cm.) ocular, given in the fourth column of the table on pages 50 and 51, are calculated for the ten inch (25 cm.) distance, and are therefore somewhat overrated practically. It should be added that the highest powers named are seldom if ever made or used; and that, to say the least, the work of most microscopists can be done more easily, if not better, with a $\frac{1}{12}$ or $\frac{1}{16}$ than with a $\frac{1}{25}$ or $\frac{1}{50}$.]

[In the table the latest published price (1884) of each lens is annexed, to show the present value of such work, and to indicate the significant relation, in the various lenses of each maker, between angular aperture and price. In a few instances other considerations overrule this law, and give an unexpectedly high or low figure. It is claimed by the makers, though often earnestly disputed by others, that the excessive prices of the high-angled lenses are not fictitious values based upon a monopoly of the supply, but they only adequately represent the amount of labor actually expended in overcoming the practical difficulties of construction. On the other hand, it is certain that the day has fortunately passed away when lowness of price

TABLE OF AMERICAN OBJECTIVES.

FOCUS.		POWER.		BAUSCH & LOMB.		GRUNOW.		GUNDLACH.		SPENCER.		WALES.		ZENT-MAYER.	
Inches	mm.	Alone	With 2 in. ocular	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $
5	127	2	10	(5-3 in.)	18			(5-3 in.)	20					(5-3 in.)	15
4	102	2½	13	6 10 12	6 13 18	12	18	8 (4-2 in.)	8 20	7 (4-2 in.)	8 20	9	12		
3	76	3½	17	9 12 16	6 13 18	12	18	11 13	8 20	8 13	7 20	12	17		
2	51	5	25	12 15 22	6 13 18	15 20 22	15 18 20	15 18	6 20	10 16 20	7 18 30				
1½	38	6½	33	16 24 32	6 15 20	15 22 30	12 18 25	18 24	6 18			23	17	23	15
1	25	10	50	20 36 45	6 15 25	25 30 50	15 20 35	15 24 26 36 85	5 12 25 20 80	25 33 40	12 22 40	25	17		
8/10	20	12	62											26 32	10 18
3/4	19	13	67	27 40	8 15										
2/3	17	15	75			25 35 65	15 22 30	25 36 40 43	6 10 15 25	32 36 47	12 22 30	30	17	32	18
1/2	13	20	100	42 65 98	9 18 30	65	18	27 40 50 72 110	7 10 20 40 50	50 n 70 100	15 25 50				
4/10	10	25	125	70 110	13 34	50 75 100	18 25 35	60 80 110	20 20 40			75 95 115	30 35 40	60 80	22 30
1/3	8	30	150			120	35								
1/4	6	40	200	n 75 100 125 na.hi 1.40	14 17 24 100	n 75	20	n 75 n 80 na.hi 1.20	14 25 60	n 100 115 13 na.di 1.28	16 30 40 70	100	25		
1/5	5	50	250	110 130	18 28	90 110 135	20 35 40	135 na.hi 1.40	22 90			135 1 170	35 40	90 120	18 35

TABLE OF AMERICAN OBJECTIVES.—*Continued.*

FOCUS.		POWER.		BAUSCH & LOMB.		GRUNOW.		GUNDLACH.		SPENCER.		WALES.		ZENTMAYER.	
Inches	mm.	Alone	With 2 in. ocular	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $	Aperture °	Cost $
1/6	4	60	300	140 i 165 na.hi 1.18 na.hi 1.40	40 23 45 70	n 100 110 165	20 25 40	n 80 na.gi 1.12 na.hi 1.20	16 30 45	di 175 na.i 1.21 na.hi 1.35	40 40 70				
1/8	3	80	400	115 135 i 170 na.hi 1.18 na.hi 1.40	21 30 25 50 75	 150	 45	 135 na.hi 1.40	 24 80	120 i 135 di 135 150 di 175	25 25 32 45 40				
1/10	2.5	100	500	i 170 na.hi 1.18 na.hi 1.40	28 50 80	i 130 i 175	30 50	 na.gi 1.12 na.hi 1.20	 25 35	na.i 1.25 na.hi 1.27 na.hi 1.35	50 60 80	135 170	35 45		
1/12	2.1	120	600	130 i 175 na.hi 1.18 na.hi 1.40	27 30 55 90	 	 	135 na.hi 1.40	40 90	 	 	 	 	 	
1/15	1.7	150	750	 	 	i 160	75	 	 	i 150 150 na.i 1.17	40 60 60	i 170	65	 	
1/16	1.6	160	800	i 175 na.hi 1.18 na.hi 1.40	35 65 125	 	 	na.gi 1.12 na.hi 1.20 na.hi 1.40	40 50 120	135 na.hi 1.35	40 125	 	 	 	
1/18	1.4	180	900	 	 	 	 	 	 	di 175	70	 	 	 	
1/20	1.3	200	1000	 	 	i 170	100	na.hi 1.20 na.hi 1.40	75 160	 	 	 	 	 	
1/25	1	250	1250	 na.hi 1.40	 200	 	 	na.hi 1.20 na hi 1.40	120 220	 na.hi 1.35	 150	160	100	 	
1/32	0.8	320	1600	 	 	 	 	na.hi 1.20	150	 	 	 	 	 	
1/50	0.5	500	2500	 	 	 	 	na.hi 1.20	200	na.hi 1.17	270	 	 	 	
1/75	0.3	750	3750	 	 	 	 	na.hi 1.20	260	na.hi 1.17	???	 	 	 	
1/100	0.2	1000	5000	 	 	 	 	na.hi 1.20	300	 	 	 	 	 	

[References in table: i = immersion; di = dry or immersion; gi = glycerine immersion; hi = homogeneous immersion; na = numerical aperture; n = non-adjustable, all lenses of over 65°, if not so marked, having screw collar adjustment for thickness of cover.]

indicated poorly finished or imperfectly corrected lenses; the cheapest objectives, by the best makers, being carefully corrected and neatly mounted, and owing their cheapness to the ease with which low-angled lenses can be corrected and the simplicity with which they may be mounted. They are therefore adequate for any work, however important, that requires the use of such angles; and they are most commonly supplied in connection with the various grades of students' and physicians' microscopes.]

[The focal distances and angular apertures in the table on opposite page are given on the authority of the several makers. There is reason to believe that these data, on account of extensive agitation of the subject, are more carefully determined and more accurately stated than formerly. The apertures given are the air-angles, unless otherwise stated. In lenses of the highest grade the numerical aperture is necessarily given, there being no corresponding air-angle. The Aperture Table of the Royal Microscopical Society, founded upon the elaborate researches of Prof. Abbe, is reproduced here as a most convenient means of comparing dry, water and homogeneous immersion lenses, and of applying the doctrine of Numerical Aperture as a measure of their theoretical illuminating, resolving and penetrating powers. For instance, 180°, the theoretical maximum of air angle, corresponds to unity of numerical aperture, and of illuminating and penetrating power; this being practically equivalent to only 97° 31′ of water angle or 82°17′ in homogeneous media corresponding to crown glass, and having a theoretical resolving capacity for 96,400 lines to the English inch. Higher apertures, as readily shown by the table, are practicable only in immersion lenses, and give increased illuminating and resolving but lessened penetrating powers; lower apertures having exactly the reverse characteristics. R. H. W.]

There is a widespread opinion that the more a microscope magnifies the better it is. This is really the case, however, only under certain conditions. A microscope which magnifies very powerfully but at the same time gives imperfect, indistinct, and poorly illuminated images is far less to be preferred than one which gives much smaller magnification but produces

APERTURE TABLE.

The "APERTURE" of an optical instrument indicates its greater or less capacity for receiving rays from the object and transmitting them to the image, and the aperture of a Microscope objective is therefore determined by the ratio between its focal length and the diameter of the emergent pencil at the plane of its emergence — that is, the utilized diameter of a single-lens objective or of the back lens of a compound objective.

This ratio is expressed for all media and in all cases by $n \sin u$, n being the refractive index of the medium and u the semi-angle of aperture. The value of $n \sin u$ for any particular case is the "*numerical aperture*" of the objective.

Diameters of the back lenses of various Dry and Immersion objectives of the same power (¼ in.) from 0·50 to 1·52 N. A.	Numerical Aperture. ($n \sin u = a$.)	Angle of Aperture (= 2 u.)			Illuminating power. (a^2.)	Theoretical resolving power, in lines to an inch. ($\lambda = 0{\cdot}5269\ \mu$ = line E.)	Penetrating power. $\left(\frac{1}{a}\right)$
		Dry objectives. ($n = 1$.)	*Water-immersion* objectives. ($n = 1{\cdot}33$)	*Homogeneous-immersion* objectives. ($n = 1{\cdot}52$.)			
	1·52	..	..	180° 0′	2·310	146,528	·658
	1·50	..	..	161° 23′	2·250	144,600	·667
	1·48	..	..	153° 39′	2·190	142,672	·676
	1·46	..	..	147° 42′	2·132	140,744	·685
	1·44	..	..	142° 40′	2·074	138,816	·694
	1·42	..	..	138° 12′	2·016	136,888	·704
	1·40	..	..	134° 10′	1·960	134,960	·714
	1.38	..	..	130° 26′	1·904	133,032	·725
	1·36	..	..	126° 57′	1 850	131,104	·735
	1·34	..	..	123° 40′	1·796	129,176	·746
	1·33	..	180° 0′	122° 6′	1·770	128,212	·752
	1·32	..	165° 56′	120° 33′	1·742	127,248	·758
	1·30	..	155° 38′	117° 34′	1·690	125,320	·769
	1·28	..	148° 28′	114° 44′	1·638	123,392	·781
	1·26	..	142° 39′	111° 59′	1·588	121,464	·794
	1·24	..	137° 36′	109° 20′	1·538	119,536	·806
	1·22	..	133° 4′	106° 45′	1·488	117,608	·820
	1·20	..	128° 55′	104° 15′	1·440	115,680	·833
	1·18	..	125° 3′	101° 50′	1·392	113,752	·847
	1·16	..	121° 26′	99° 29′	1·346	111,824	·862
	1·14	..	118° 00′	97° 11′	1·300	109,896	·877
	1·12	..	114° 44′	94° 56′	1·254	107,968	·893
	1·10	..	111° 36′	92° 43′	1·210	106,040	·909
	1·08	..	108° 36′	90° 33′	1·166	104,112	·926
	1·06	..	105° 42′	88° 26′	1·124	102,184	·943
	1·04	..	102° 53′	86° 21′	1·082	100,256	·962
	1·02	..	100° 10′	84° 18′	1·040	98,328	·980
	1·00	180° 0′	97° 31′	82° 17′	1·000	96,400	1·000
	0·98	157° 2′	94° 56′	80° 17′	·960	94,472	1·020
	0·96	147° 29′	92° 24′	78° 20′	·922	92,544	1·042
	0·94	140° 6′	89° 56′	76° 24′	·884	90,616	1·064
	0·92	133° 51′	87° 32′	74° 30′	·846	88,688	1·087
	0·90	128° 19′	85° 10′	72° 36′	·810	86,760	1·111
	0·88	123° 17′	82° 51′	70° 44′	·774	84.832	1·136
	0,86	118° 38′	80° 34′	68° 54′	·740	82.904	1·163
	0·84	114° 17′	78° 20′	67° 6′	·706	80,976	1·190
	0·82	110° 10′	76° 8′	65° 18′	·672	79.048	1·220
	0·80	106° 16′	73° 58′	63° 31′	·640	77.120	1·250
	0·78	102° 31′	71° 49′	61° 45′	·608	75,192	1·282
	0·76	98° 56′	69° 42′	60° 0′	·578	73,264	1·316
	0·74	95° 28′	67° 36′	58° 16′	·548	71,336	1·351
	0·72	92° 6′	65° 32′	56° 32′	·518	69.408	1·389
	0·70	88° 51′	63° 31′	54° 50′	·490	67,480	1·429
	0·68	85° 41′	61° 30′	53° 9′	·462	65,552	1·471
	0·66	82° 36′	59° 30′	51° 28′	·436	63,624	1·515
	0·64	79° 35′	57° 31′	49° 48′	·410	61,696	1·562
	0·62	76° 38′	55° 34′	48° 9′	·384	59,768	1·613
	0·60	73° 44′	53° 38′	46° 30′	·360	57,840	1·667
	0·58	70° 54′	51° 42′	44° 51′	·336	55,912	1·724
	0·56	68° 6′	49° 48′	43° 14′	·314	53,984	1·786
	0·54	65° 22′	47° 54′	41° 37′	·292	52,056	1.852
	0·52	62° 40′	46° 2′	40° 0′	·270	50,128	1.923
	0·50	60° 0′	44° 10′	38° 24′	·250	48,200	2·000

EXAMPLE.—The apertures of four objectives, two of which are dry, one water-immersion, and one oil-immersion, would be compared on the *angular* aperture view as follows:—106° (air), 157° (air), 142° (water), 130° (oil).

Their actual apertures are, however, as ·80 (air), ·98 (air), 1·26 (water), 1·38 (oil), or their numerical apertures.

Scale showing the relation of Millimeters, etc., to Inches.

1000 μ = 1 mm.
10 mm. = 1 cm.
10 cm. = 1 dm.
10 dm. = 1 m.

Micromillimeters, etc., into Inches, etc.

μ	ins.	mm.	ins.	mm.	ins.
1	·000039	1	·039370	51	2·007892
2	·000079	2	·078741	52	2·047262
3	·000118	3	·118111	53	2·086633
4	·000157	4	·157482	54	2·126003
5	·000197	5	·196852	55	2·165374
6	·000236	6	·236223	56	2·204744
7	·000276	7	·275593	57	2·244115
8	·000315	8	·314963	58	2·283485
9	·000354	9	·354334	59	2·322855
10	·000394	10 (1 cm.)	·393704	60 (6 cm.)	2·362226
11	·000433	11	·433075	61	2·401596
12	·000472	12	·472445	62	2·440967
13	·000512	13	·511816	63	2·480337
14	·000551	14	·551186	64	2·519708
15	·000591	15	·590556	65	2·559078
16	·000630	16	·629927	66	2·598449
17	·000669	17	·669297	67	2·637819
18	·000709	18	·708668	68	2·677189
19	·000748	19	·748038	69	2·716560
20	·000787	20 (2 cm.)	·787409	70 (7 cm.)	2·755930
21	·000827	21	·826779	71	2·795301
22	·000866	22	·866150	72	2·834671
23	·000906	23	·905520	73	2·874042
24	·000945	24	·944890	74	2·913412
25	·000984	25	·984261	75	2·952782
26	·001024	26	1·023631	76	2·992153
27	·001063	27	1·063002	77	3·031523
28	·001102	28	1·102372	78	3·070894
29	·001142	29	1·141743	79	3·110264
30	·001181	30 (3 cm.)	1·181113	80 (8 cm.)	3·149635
31	·001220	31	1·220483	81	3·189005
32	·001260	32	1·259854	82	3·228375
33	·001299	33	1·299224	83	3·267746
34	·001339	34	1.338595	84	3·307116
35	·001378	35	1·377965	85	3·346487
36	·001417	36	1·417336	86	3·385857
37	·001457	37	1·456706	87	3·425228
38	·001496	38	1·496076	88	3·464598
39	·001535	39	1·535447	89	3·503968
40	·001575	40 (4 cm.)	1·574817	90 (9 cm.)	3·543339
41	·001614	41	1·614188	91	3·582709
42	·001654	42	1·653558	92	3·622080
43	·001693	43	1·692929	93	3·661450
44	·001732	44	1·732299	94	3·700820
45	·001772	45	1·771669	95	3·740191
46	·001811	46	1·811040	96	3·779561
47	·001850	47	1·850410	97	3·818932
48	·001890	48	1·889781	98	3·858302
49	·001929	49	1·929151	99	3·897673
50	·001969	50 (5 cm.)	1·968522	100 (10 cm. = 1 dcm.)	
60	·002362				
70	·002756				
80	·003150				
90	·003543				
100	·003937				
200	·007874				
300	·011811				
400	·015748				
500	·019685				
600	·023622				
700	·027559				
800	·031496				
900	·035433				
1000 (= 1 mm.)					

decim.	ins.
1	3·937043
2	7·874086
3	11·811130
4	15·748173
5	19·685216
6	23·622259
7	27·559302
8	31·496346
9	35·433389
10 (1 meter)	39·370432
=	3 280869 ft.
=	1·093623 yds.

Inches, etc., into micromillimeters, etc.

ins.	μ
$\frac{1}{25000}$	1·015991
$\frac{1}{20000}$	1·269989
$\frac{1}{15000}$	1·693318
$\frac{1}{10000}$	2·539977
$\frac{1}{9000}$	2·822197
$\frac{1}{8000}$	3·174972
$\frac{1}{7000}$	3·628539
$\frac{1}{6000}$	4·233295
$\frac{1}{5000}$	5·079954
$\frac{1}{4000}$	6·349943
$\frac{1}{3000}$	8·466591
$\frac{1}{2000}$	12·699886
$\frac{1}{1000}$	25·399772
	mm.
$\frac{1}{900}$	·028222
$\frac{1}{800}$	·031750
$\frac{1}{700}$	·036285
$\frac{1}{600}$	·042333
$\frac{1}{500}$	·050800
$\frac{1}{450}$	·056444
$\frac{1}{400}$	·063499
$\frac{1}{350}$	·072571
$\frac{1}{300}$	·084666
$\frac{1}{250}$	·101599
$\frac{1}{200}$	·126999
$\frac{1}{150}$	·169332
$\frac{1}{100}$	·253998
$\frac{1}{50}$	·507995
$\frac{1}{25}$	1·015991
$\frac{1}{20}$	1·269989
$\frac{1}{16}$	1·587486
$\frac{1}{15}$	1·693318
$\frac{1}{12}$	2·116648
$\frac{1}{10}$	2·539977
$\frac{1}{8}$	3·174972
$\frac{1}{6}$	4·233295
$\frac{3}{16}$	4·762457
$\frac{1}{5}$	5·079954
$\frac{1}{4}$	6·349943
$\frac{5}{16}$	7.937429
$\frac{3}{8}$	9·524915
	cm.
$\frac{7}{16}$	1·111240
$\frac{1}{2}$	1·269989
$\frac{9}{16}$	1·428737
$\frac{5}{8}$	1·587486
$\frac{11}{16}$	1·746234
$\frac{3}{4}$	1·904983
$\frac{13}{16}$	2·063732
$\frac{7}{8}$	2·222480
$\frac{15}{16}$	2 381229
1	2·539977
2	5·079954
3	7·619932
	decim.
4	1·015991
5	1·269989
6	1·523986
7	1·777984
8	2.031982
9	2·285979
10	2 539977
11	2·793975
1 ft.	3·047973
	meters.
1 yd. =	·914392

clear and sharp images. It may be said, on the contrary, that those instruments are comparatively the best which, with relatively low magnifications, still show the details which appear in poor instruments only with the use of high powers.

In judging of a microscope the first things to be thought of are these: Does it show a sharp and clear image, all details, all fine structural relations? What are its defining and resolving powers? Then, one may inquire how many times it magnifies. We shall now proceed to show by a simple method how one may satisfactorily judge of the defining and resolving powers of a microscope.

VII. TESTING THE OPTICAL POWERS.

The defining and resolving powers of a microscope may be best tested by means of so-called "proof objects" or "test objects." These consist of small parts of animals or plants, and also of very small whole organisms which are prepared in a certain way. They should be examined with a known magnification, which should be produced mainly by the objective. It should then be ascertained if the image appears the same in this as in some notoriously good microscope, or comparison should be made with some distinct and clear illustration or exact description which may be accessible. If, then, one recognizes all those details which the illustration or description, with a like magnification, furnishes, it is a sign of the good quality of the microscope. But if the outlines of the form and the fine structural relations are more indistinct than in the illustration, or if they are in general not visible, it would thence follow that the instrument would not fully satisfy modern requirements.

Since the testing of the optical performance of a newly acquired instrument is the first thing to be taken in hand, the maker usually takes care to provide some test-object by which this may be done.

Testing the instrument, particularly with objects of difficult resolution, should take place on a not too dark day, when the heavens are uniformly covered with a veil of transparent clouds;

at all events, not when the sky is filled with numerous gray, rapidly moving clouds which produce constant changes in the light. The microscope should be placed close before an open window facing the north or east. The illumination of the object should be by central light from the mirror, oblique light being in almost all cases unsteady. The size of the aperture in the diaphragm under the stage to be employed will depend upon the power of the objective in hand. It is also recommended that people who use spectacles should lay them aside when observing test objects, since the least particle of greasy matter, such as might easily be left on the glass by the motion of the eyelashes, would render the microscopic image less sharp and clear. It is self-evident that the objectives, oculars and the glasses holding the test-object should be perfectly clean.

We will first discuss those objects by which we test the defining power and afterwards those by which we test the resolving power of the microscope.

A. Testing the Defining Power.

All the objects used for this purpose in the magnifications employed must show an altogether distinct, clear, delicate and colorless outline. The test-objects used exclusively for definition should be employed only with low or medium powers, while those for resolution should be used with the highest powers, they likewise giving at the same time tests of the definition. The most important tests for definition are the following:

1. *The Calcareous Plates of the Synapta.* That part of the echinoderm group known as Holothurians or "sea-rolls" contains animals of a mostly cylindrical form whose bodies are covered with a leathery skin. Imbedded in this skin are a great number of microscopically minute, perfectly colorless (rarely somewhat colored) calcareous bodies. The genus *Synapta*, of which representatives are found in the Mediterranean, but whose more numerous species occur in the warm seas of Polynesia and southern Asia, shows an extraordinarily delicate example of these small, calcareous bodies. The integument of these long

worm-like animals is pretty thin, and growing in it are rectangular or roundish, perforated, calcareous plates (Plate I, Fig. 1, *b*) in which anchor-like hooks are fastened (Plate I, Fig. 1, *a*).[30] The fully formed calcareous anchors have a length of 0.97–0.98 mm., are as clear as glass and perfectly transparent. The end of the handle is slightly bifurcated and a little warty-rough. The anchor hooks are curved bow-like and bluntly rounded at the end. The rectangular little plates are about 0.8 mm. long, and are perforated with from 34 to 40 holes great and small, the larger being in the middle and the smaller towards the ends.[31]

The anchors of the *Synapta* are suitable for testing the resolving power of very low objectives only (10 to 50 magnifications). It should show a contour in this magnification sharply bounded by a thick black line. In the perforated plates this contour, corresponding to its greater thinness, is more delicate than in the anchors. Poor glasses show an imperfectly defined outline with a soft haze around the edges. Lenses imperfectly achromatic produce a numerously colored border near the edges. The illustration in Plate I, 1, is prepared from a magnification of about thirty diameters. The specimen should be mounted in balsam.

2. *Transverse section of Coniferous wood.* While the objects already described are suitable only for testing the lower powers, we have, in a transverse section of the needle-bearing trees, a very good object for testing the definition of medium, and even stronger powers. It is a matter of indifference which one of the coniferous species one chooses from which to make the preparation. The one illustrated here, Plate I, Fig. 2, is a piece of the cross section of the young stem of the common fir tree (*Pinus sylvestris*) which may be found anywhere. The preparation was made in this way. The most delicate possible section was made through the young stem. No dull places in the knife should be permitted to leave their marks upon the

[30] The calcareous bodies from the following and other species of the *Synaptae* are suitable for test-objects. *Synapta inhaerens, glabra, Godefroyi, recta, similis, molesta, Kefersteinii, Besselii, digitata.*

[31] There are also species (for example, *S. inhaerens*) in which the calcareous plates have a few large holes with toothed edges.

section. How this is to be avoided will be shown more exactly hereafter. The section should then be put for some minutes in absolute alcohol to remove the resin from the resin tubes,[32] then it should be washed in distilled water and mounted in glycerine-jelly or glycerine.

For our purpose we shall consider the wood layer which forms one concentric ring. With high magnification the wood cell[33] presents the three following layers in its walls. The one layer which is common to the two cells, and which we shall with Sachs name the "middle layer," *a*, is thin and highly refractive. Then follows toward the inside a second stronger layer, which is compounded from several concentric lamellæ, the intermediate thickening layer *b*, and upon this lies a soft layer which lines the inside of the cell, and is called the inner thickening layer, *c*. All these layers should appear clearly and sharply differentiated from each other with a linear magnification of from 450 to 800 diameters. The middle layer is commonly somewhat easier to see than the inner thickening layer. Hence the former will become visible earlier when the preparation is put under the lens, and the latter as it is progressively subjected to higher and higher powers. The inner layer is best adapted to give account of the definition of high power lenses. With good objectives the inner layer should show itself separated from the cell cavity in sharp outline, a very sharp delicate line bounding it. Poor objectives, on the other hand, show a broad gray line about the cell cavity which becomes clearer inwards, with no sharp limits but gradually loses itself in a delicate haze. It should be noticed that thick and imperfect sections produce the same kind of an image. On this account, only the thinnest possible sections, as already mentioned, should be used for this investigation.

Longitudinal sections of the woody parts of this plant furnish good test-objects for these magnifications and for somewhat lower powers. Here we find the great rounded "bordered pits"[34] which are very suitable for testing the definition of an

[32] Sach's Lehrb. der Bot., IV, Aufl. p. 95, Fig. 78.
[33] Sachs, *l. c.*, p. 75, Fig. 57.
[34] Sachs, *l. c.*, p. 25, Fig. 23.

objective. Their entire outline should appear sharp and clear, as simple lines.[35]

3. *Scale-dots of Lycaena.* We may for the same purpose, with good results, employ the scales of the "Bluelings." I indeed recommend that the three species be used in the investigation: *Lycaena Alexis* F. (=*Icarus* Hbst.), *L. argiolus* L. or *L. argus* L. As with all butterflies their wings are closely covered with numerous small stalked scales, to which they are indebted for their lively colors. On the upper as well as on the under side of the wing are two kinds of scales, one of a longer form which bears on its surface delicate longitudinal markings, and another which consists of a longer style joined to an elliptical plate. The latter are provided on the upper surface with a few (6 to 10) dotted longitudinal markings, Plate I, Fig. 3. These scales should be mounted in Canada balsam for test-objects. Good medium objectives magnifying 350 to 450 diameters should show in the former kind the longitudinal markings clearly as double lines. And in the latter the dots should appear as small circles which have a minute dark dot in the middle. The markings and the dots should not mingle or blend with each other. The specimen is somewhat more difficult to resolve when mounted dry, than in Canada balsam, and appears colored.

B. Testing the Resolving Power.

In order to be assured of the superior quality of the higher and highest power objective-systems we must employ the best test-objects, which in the first instance permit us to judge of the resolving power, and at the same time are a test of the definition. Both qualities of the objective are essentially bound up together. An objective-system with unsatisfactory definition will never resolve difficult images. But it sometimes happens that two systems of very nearly the same resolving power, showing the resolved details in sharper or fainter outlines, do not possess

[35] Preparations of potato starch grains may be employed as tests of definitions for medium powers. They should be examined in water or glycerine, and their separate layers, which are grouped about an excentric formation-point, should be bounded by a sharp, strong and delicate outline. (See Sachs, *l. c.* Fig. 51, on p. 62.)

the same power of definition. The test-objects most often used for resolution are butterflies' scales and the siliceous frustules of different species of diatoms.

1. Scales of *Hipparchia janira* and *Lycaena argiolus*. Our common white butterfly, *Hipparchia janira*, has on its wings several kinds of scales, short, medium and long. A scale of medium length is illustrated in Plate I, Fig. 4. It has a breadth of 0.059 mm., and a length of 0.156 mm. It is rectangular, has three broad points at top, and is heart-shaped at bottom, ending in a short style. Its surfaces are covered with 22–24 longitudinal ridges, which have an average distance apart of 0.00266 mm., so that about four of these go to 0.01 mm. ($10\mu = 10$ micromillimetres). Magnified as in the illustration (305 diameters) we see very many delicate cross lines between the longitudinal elevations. The higher the magnification, the more the finer details are brought out, and the butterfly's scale therefore furnishes a very excellent preparation for testing the resolving power of the strongest objective-system. In recent times, Dippel[36] has most accurately investigated the butterflies' scales employed as test-objects, and we shall give here in his own words the results to which that naturalist has arrived in his very exact studies of these important tests. "The longitudinal flutings are made by the elevation of the upper surface, between which run furrow-like depressions so that the scale seen in section has a wavy aspect. When viewed with lower powers, and oblique light falling upon them in a direction perpendicular to their length, they appear to be bounded by two sharp lines. With higher magnification and central light, and with an objective of good definition, they assume a toothed appearance, and because of the cross-lines which lie in the same plane, and come into the focus at the same time, they take the appearance of being thickened at these points. With the lower powers, this structural relationship, on account of its delicacy, shows with scarcely half the sharpness of the boundary lines. With oblique illumination, even with the medium higher powers, it is overlooked, because the shadows cast by the longitudinal ridges apparently obscure

[36] Dippel, *l. c.*, p. 118*f.*

these fine lines. Herein we see the foundation for the widely divergent views which microscopists have expressed about the real nature of these longitudinal markings. Thus, for example, Brewster (Treatise on the Microscope) declared that the cross markings did not exist at all, but that the longitudinal ridges were beset with small teeth. Chevalier (Les Microscopes, etc.,) described the scales of *Pieris brassicae* as beset with longitudinal ridges which were formed by minute balls placed near each other, and held that the true test of an objective-system consisted in making these globules visible. Some English micrographers agreed with this. Others, for example, Goring, then under the German H. v. Mohl, controverted it, and asserted the existence of sharply defined longitudinal and transverse markings, and held Chevalier's description of this test-object to be a plain witness that the latter had misinterpreted his microscope. Brewster is only partly in the right with his statement, since he quite overlooked the cross-lines, or rather failed to see them altogether; but Chevalier decided correctly. I have examined the same object anew, with several of the best objective-systems of recent times and find them formed as Chevalier asserted. The well-known heart-shaped scales of *Pieris brassicae*[37] are, especially over their upper surfaces, both on the longitudinal markings and intervening spaces, beset with small, irregularly angled, or roundish bodies, whereby, under certain conditions of illumination the appearance of cross-lines is produced, which run between and near the longitudinal lines, but which by direct illumination and good defining objectives are seen to appear in the way pointed out by Chevalier.

In most scales, the cross markings run in a direction perpendicular to the axis of the longitudinal ridges; in others, in an oblique direction, as well over the summits of them, as across the intervening spaces, without interruption. In those adjustments of the microscope, however, with which one commonly chooses to see the cross marking between the others with distinctness, these facts easily escape observation. Only by a certain medium adjustment do they come forth clearly. The

[37] See Dippel, *l. c.*, Figs. 61 and 62, on p. 119.

cross markings are not like the longitudinal, elevations, but rather depressions between the more or less regularly rectangular to roundish bodies, which as a rule stand in series of four between each two of the longitudinal markings. Thence between each two of the stronger longitudinal markings, there are three others very much more delicate, and which are far more difficult to see than the transverse markings, and afford good tests of the strongest lenses. The best objective-system in which is united the greatest resolving power with the best definition as well as the most perfect chromatic correction, can alone make us acquainted with this structure.[38] Bruno Hasert had already, in 1847, traced out this structure and since then more fully examined it. (Official report of the 34th meeting of German Naturalists and Physicians, at Karlsruhe, p. 212.) At his suggestion, I have likewise most carefully examined this object with my most powerful objectives, and have convinced myself definitely of the correctness of his representation. Thus we have established a solid footing as to how these cross markings ought to look through good objectives. In the same way we explain the diagonal markings on these scales, which one perceives with certain illuminations and with strong objectives. If we examine the transverse lines with direct light and a magnification of 300 to 500 diameters they will appear as if serrated, but they must be sharply defined for that. With higher magnifications the separate little bodies will appear with clear delicate outlines, as soon as the spherical aberration of the objective is perfectly corrected. Oblique illumination, on the contrary, is the cause of obscuring the true structure with low magnifications, and affords sharply marked linear cross stripes, as they have been heretofore represented by those microscopists who have in such testing worked with their mirrors excentrically placed. Objectives also which are not strictly first-class with respect to definition, or have imperfect correction of chromatic aberration will give that kind of an image, with high magnifications. On this we may ground objections to a system which is known to be otherwise excellent, that it shows the cross markings of the *Hipparchia* scale as serrated. On the

[38] See Plate I, Fig. 7, which represents a copy of Dippel's, Fig. 69.

contrary, we must accept it as evidence of the good quality of an objective, that it shows these markings with central illumination quite distinct and sharp, while one which does not thus show them betrays a lack of defining power. The diagonal lines come out when the oblique light falls upon the scale at an angle of 30° to 60° to the axis of the longitudinal markings, while the delicate longitudinal lines come out most clearly when the oblique light falls perpendicularly upon the longitudinal axis. In respect to the visibility of the two last named systems of lines, the butterflies' scales furnish test-objects of a difficulty almost equal to the diatoms, without, however, affording a sufficiently perfect series of comparisons."

It should be added that Plate I, Figs. 5, 6, and 7, shows us single pieces of the scale of *Hipparchia janira* under different magnifications, Fig. 5 × 500, Fig. 6 × 1450, Fig. 7 × 1920 times, the last two after Dippel.[39]

Lycaena argiolus has scales which are somewhat more difficult to resolve than those of *Hipparchia janira* in respect to their corresponding fine markings. Fig. 8, Plate I, represents a whole scale × 305 times. Fig. 9, a piece × 500, and Fig. 10, a small piece × 1450 times (after Dippel).[40]

Butterfly scales are commonly mounted in Canada balsam for test-objects. Mounted dry they are somewhat more difficult. Glycerine mounting seems to me very serviceable also.

2. *The Siliceous frustules of the diatoms.* There occurs in the mire and on plants in stagnant waters, and also in the sea, a group of single-celled algæ in an almost endless variety of forms and species, known in general as diatoms. They are throughout of microscopical minuteness, and are distinguished from all other algæ by having their cell walls covered with a framework of pure silex, which consist of frustules that fit upon each other and represent a perforated lattice-work of the most delicate structure. This siliceous shield consists of two separable halves. If the diatoms are boiled in a mixture of potassium chlorate and nitric acid the entire organic substance will be destroyed and only the siliceous frames will be left over

[39] Dippel, *l. c.*, Figs. 63 and 69, on p. 119 and 122.
[40] Dippel, *l. c.*, Fig. 73, on p. 123.

with the two halves parted. These separated halves of the frustules are what furnish the most excellent test-objects. They are prepared in two ways, either mounted dry or in balsam, and the method of examination as test-objects is determined by the particular kind of mounting. On the whole, those mounted in balsam are more difficult to resolve than those mounted dry.

Dry mounts are most suitably made as follows. After the diatoms have been separated by the mixture of nitric acid and concentrated solution of potassium chlorate, the fluid containing the diatoms is put into a high and narrow test tube, and the diatoms are allowed to settle to the bottom. They are then repeatedly washed in distilled water till litmus paper no longer shows any trace of the acid. A sample of the diatomiferous fluid is taken out with a pipette, and placed on a clean slide, and the water allowed to evaporate in some place free from dust, (for example in a drying chamber) and a cover glass cemented on over it, as will be described further along. The balsam mount is made in the following way. A small portion of the cleaned diatomiferous fluid is put in a watch glass and evaporated. The residue is mixed with pure oil of turpentine, and then a drop of the turpentine oil with the suspended diatoms is mingled with a drop of Canada balsam on a slide; lay on a cover glass and fasten with gentle warming.[41]

Several species of the genus *Pinularia* make tests for very low powers. The cross markings on the long sausage-shaped frustules of *Pinularia nobilis* Eh. can be easily and clearly seen with a magnifying power of thirty diameters, but the like formations on the elongated elliptical body of *P. viridis* Rh. must be magnified 209 diameters to be distinctly seen. Since we have shown how the butterfly scales may most suitably test the

[41] Except in rare cases, we shall not ourselves undertake the preparation of the diatom test-objects. They can be had at the best microscopical institutes (Dr. Kaiser in Berlin, Möller in Wedel, Holstein, Rohdig in Hamburg, etc.) [In America all of the principal dealers in microscopical goods keep diatom tests on sale, and I believe Mr. C. L. Peticolas of Richmond, Va., has made a specialty of the preparation of these diatom test-objects, A. B. H.] at the price of one to two Marks each (25 to 50 cts.) Möller furnishes a diatom test plate which contains under the same cover-glass, a number of diatom frustules for test-objects so arranged that those at one end are easiest, and they become progressively harder of resolution towards the other end. so that it is possible to test the power of resolution of all systems by means of this one preparation.

lower powers, we need not further consider the diatoms as tests for objectives of that kind.

For objective-systems of medium magnifications, the various species of the genus *Pleurosigma* make most extraordinarily beautiful test-objects. They are almost all good tests also for the higher powers. The species of the Pleurosigma are easily recognized by the peculiar sigma-like (ς) curvature of their bodies. A doubly curved central line is drawn along their whole length which in the middle is expanded into a lengthened nodule. On each side of the central line, the frustule is covered throughout with a framework of delicate lines, which now lay claim to our exclusive attention. The species used as test-objects, may be separated into two groups according to the construction of the above mentioned framework or skeleton. The first includes the species *Pleurosigma balticum* and *Pl. attenuatum;* to the second belong *Pl. angulatum* and *Pl. formosum.*

(*a*) We shall first consider *Pleurosigma balticum* Sm. This little plant is from 0.29 to 0.33 mm. long, and should be mounted in Canada balsam when used as a test object. Plate I, Fig. 11–14. By a magnification of 100 diameters the object appears as a delicate hyaline form, of the shape of Fig. 11. We distinguish the lateral boundary edges, and the beautifully curved central line with a knot in the middle. With this magnification we may perceive either by central or oblique light, minute carvings on the surface of the frustules. If the magnification be now raised to 200 diameters, by means of a stronger objective and the weakest possible ocular, the surface will appear to be covered with a very delicate lattice-work. We see at once that this consists of longitudinal and transverse *striæ*. By different focussing, now the former, and now the latter, will be made distinctly visible, particularly towards the outer edges. Now, if we choose a higher ocular, taking the same objective, and make the magnification 300 diameters, the image will become a little clearer since the single lines will be farther apart. Two transverse lines stand at a distance of 0.0007 mm., so that about fifteen of them go to make 0.01 mm. An objective magnification of about 460 clearly resolves the lattice-work into two systems of lines which stand perpendicu-

lar to each other, one running lengthwise and the other across the diatom. We now without difficulty see that at the intersecting points of the *striæ* of the two systems are knot-like thickenings, Plate I, Figs. 12, 13, the form of which is apparently rectangular.

A little stronger ocular magnification (550 to 590) Fig. 12, 13, makes the image still somewhat more distinct. Finally, the markings appear to be perfectly resolved with a magnification of 950, Plate I, Fig. 14. The knots have now clearly a four-sided form, and by a still stronger magnification (1400 to 1450) we recognize the fact that they are in reality six-sided, but the six angles are not regular, two opposite sides being shorter than the other four.[42]

(*b*) *Pleurosigma angulatum* Sm. forms the diatom test which is properly most in use for medium and higher powers. They should be mounted dry. If mounted in Canada balsam, they are considerably more difficult of resolution, and the following description would not apply to a balsam mount. *Pl. angulatum*, which may easily be distinguished from all other species of the genus by its form, attains a mean length of 0.24 to 0.32 mm. Both sides of the middle portion of the frustule are drawn out somewhat sharply angular, Plate II, Fig. 1, whence it gets its name. The middle rib differs but little from *Pl. balticum* only being a little slenderer and lacking the slight curvature each side of the central nodule. With low magnification *Pl. angulatum* has a bright yellow-brown color. We shall now examine the structure of the frustule of this diatom with low, medium, higher and highest magnification.

1. *Low powers* (50–150 diameters). The surface shows a perfectly homogeneous aspect. The color is uniformly clear

[42] To those who are disposed to undertake the testing of microscopes for themselves by means of these objects, we especially commend the work of Fritsch and Müller. "The carvings and finer structural relations of the *Diatomaceæ*, with reference to the use of the species as test-objects. Part I, 12 plates, photo-micrographic illustrations, Berlin, 1870, 4to. Price 16 Marks, each plate singly 1.6 Marks." The plates represent beautiful photographs direct from the microscope: I. Diatom type plate, No. II of J. D. Möller in Wedel, magnified 90 diameters.— II. *Arachnodiscus ornatus* Ehrbg. × 530.— III. *Triceratium favus* Ehr. × 545.— IV. *Pinularia nobilis* Kg. × 545.— V. *Navicula Lyra* Ehr. and var. × 530.— VI. *Stauroneis Phœnicenteron* Ehr. × 545.— VII. *Pleurosigma balticum* Sm. × 545.— VIII and IX. *Pl. angulatum* Sm. × 545, 1200.— X. *Grammatophora marina* Sm × 545.—*Gram. oceanica* Ehr. = *G. subtilissima;* × 700.— XI., XII. *Surarella gemma* Ehr.; × 700, 1200.

brown. Oblique illumination reveals no further details on the surface. The diatom cannot be used as a test for lower powers.

2. *Medium powers* (200–400 diameters). With a magnification of 200 to 250 diameters, produced in conjunction with the weakest ocular, we first recognize traces of markings, Plate II, Fig. 1, which come forth in a dark brownish shade. Concerning the nature of these markings, this magnification permits us to say nothing further. But if one raises the magnification by a somewhat stronger ocular, to about 300 diameters, and uses oblique illumination, the markings will be distinct, and recognized as consisting of three systems of *striæ*, which are inclined to each other at an angle of about 120°. With this magnification, however, with the use of oblique illumination, we recognize these systems only when the light falls upon them severally at an angle of 90°. Concerning this quality which it is well to regard, Dippel[43] first remarked: "If the oblique light be applied to one side, there will appear one or the other of the systems of lines according to the situation of the diatom. When the oblique light falls parallel with its longest axis the somewhat more widely separated cross-lines will appear. If now we turn the light about 90°, the more closely drawn diagonal systems of lines will appear, with somewhat the same sharpness. On the contrary, only one of these systems of lines will become sharply visible when the longitudinal axis of the frustule forms an angle of about 45° with the direction of the rays." Condenser illumination, without stopping off the central rays, gives on the whole a much clearer picture than the above described process.

3. *Higher magnifications* (450 to 900 diameters). When the objective magnification reaches 450 to 480 diameters, the three systems of lines on the surface of the frustule become distinctly visible, mutually crossing each other at an angle of 120°, Plate II, Fig. 2. But we next observe that what at first appeared to be *striæ* are not straight lines, but that they are hexagons lying side by side in rows in the same direction, which cover the whole surface of the diatom with the most extraordinarily delicate lattice-work, Plate II, Fig. 4. With a magnifi-

[43] Dippel, Das Mikroskop. Bd. I, p. 128.

cation of 450 to 480 diameters, the hexagons can be seen distinctly only when the light is central and the focussing upon a given place is extremely sharp. If the illumination is excentric some one system will prevail, according to the direction in which the lattice-work is illuminated, as is indicated above.[44] Especially, with central condenser illumination, the image becomes very clear. By right focussing, the little hexagonal surfaces will appear quite colorless, and their sharply bounded contour somewhat chocolate colored.[45]

When the image is produced with a good objective, it ought to bear considerable ocular magnification without impairing its distinctness. Produced with a good Gundlach's immersion-system, VIII, the image bore an ocular magnification of 1000 to 1400 diameters, still showing the corners of the hexagon perfectly angled and sharply defined.

4. *The highest magnifications* (900–2000 diameters). With magnifications of over 900 the resolution of the hexagonal network will become still more distinct. Since the true diameter of the hexagon is about 0.005 mm., it is evident that it may be very distinctly perceived when magnified, we will say, to 1000 times. We have repeatedly studied *Pl. angulatum* with the Seibert immersion-system, No. IX (× 950, 1430, 2170, 2880), and have seen its minutest structure with central and oblique illumination and with the use of all oculars; and most beautifully by the use of a condenser with the middle rays shut out. Plate II, Fig. 4, shows the image with a magnification of 2880 with the above mentioned system; Fig. 3, with a linear enlargement of 1200, the latter from the photo-micrograph of Fritsch, Plate IX.

The species of the genera *Grammatophora* and *Nitzschia* are connected with those of *Pleurosigma* in affording the very best test objects for the higher and highest powers of the microscope, as is also *Surirella gemma* whose delicate siliceous frustule can be resolved only by the highest and best magnification.

[44] This unequal prevalence shows very well in Plate VIII of the above cited work of Fritsch and Müller which represents a *Pleurosigma angulatum* with excentric illumination. The photograph should be examined in different places with a good magnifying glass.

[45] By wrong focussing, exactly the contrary effect is produced; the surfaces are dark and the contour bright.

(*c*) *Grammatophora marina* Sm. The body of the *Grammatophora* species can be compared, as to its form, with nothing better than a cigar case. The exterior aspect of this genus of diatoms presents the form of a more or less elongated rectangle with the angles blunt or rounded, Plate II, Figs. 5, 7. Next to the middle, run two zigzag-like bent, coarse lines, whose form and position vary according to the species. Outside of these longitudinal lines, on the whole extent of the outer border, runs a zone which is filled with the most delicate transverse *striæ*. These exclusively claim our attention here.

If we examine the *Gr. marina* (in Canada balsam) with a magnification of 200 diameters, the cross lines spoken of are scarcely visible. We shall see them only after looking a long time. The markings become somewhat more distinct, with the use of excentric illumination, or better still with condenser illumination. The weaker ocular magnifications change the image but very little, but if it be raised to about 600 diameters, and excentric illumination be used, every cross line will appear to consist of a clear bright and a dark or shadowed line.

If now we raise the objective magnification to 450–480 diameters, the markings become very distinct, both by central and central-condenser illumination. Light which falls upon them obliquely, and especially in a direction perpendicular to the markings, brings out the image much more distinctly. With central illumination and very high focussing, the striated part of the diatom appears to be covered with minute points; deeper, the cross lines seem to be granulated. The image is most distinct when the focus is made to touch the middle between the upper and lower surface of the diatom.[46] Fig. 6 represents *Gr. marina* magnified 600 diameters.

Stronger magnifications (900 to 1200) change the image but

[46] After a very exact study of the *Grammatophora* species, I here express the view that the transverse *striæ* are formed of a series of granular elevations standing one behind another. In this I come into controversy with Dippel who supposes that besides the smooth transverse striation, there are other systems of lines which lie over these. "In all other species, there occurs, as in *Pl. angulatum*, along with the transverse *striæ*, another diagonal system of lines which cross these, and which in *Gr. subtilissima* are extremely difficult to see, and for this resolution it is necessary to have the most favorable illumination by the use of well regulated oblique light." (Dippel, *l. c.*, Bd. I, p. 129.) I hold on these grounds that Dippel's illustrations, Figs. 87-90, are not altogether true to nature. Compare them with Fritsch and Möller, *l. c.*, Plate X.

little. *Gr. marina* is principally recommended as an object for the weaker immersion systems.

(*d*) *Grammatophora oceanica* Ehrbg.=*Gr. subtilissima*. This species is distinguished from the one described above by its differing form (it is slenderer and longer than *Gr. marina*) and by the considerably greater fineness and difficulty of resolution of the transverse *striæ*. While the transverse lines of *Gr. marina* stand about 0.00041 mm. apart, those of *Gr. oceanica* are removed but about 0.00031 mm. from each other. An objective magnification (dry lens) of 200 diameters does not resolve the markings in the least, neither by the use of central, oblique, or condenser illumination, nor even if the magnification be raised by means of oculars to 400 or 500 diameters. The same thing happens with the lowest immersion system whose lowest ocular magnification does not exceed 400 or 500. It is only when the magnification reaches 700 with a low ocular that the cross bars appear in the form of very delicate lines, Plate II, Fig. 7. With still higher immersion systems, 1200 to 1500 diameters, they appear still more distinct and differ in no important way from those of the species described above. Fig. 8 shows this in a balsam preparation.

(*e*) We may briefly make mention here of a very good test-object from another genus of diatoms, *Nitzschia linearis*, Plate II, Figs. 9, 10. It has a peculiar wand-like form, deeply channelled on the outer edge. Proceeding from this there are drawn over the whole surface transverse lines, which in respect to delicacy, and distance from each other, stand between the two species of *Grammatophora* (0.00036 mm.). A clear resolution of these can be reached only by the strong immersion-systems. Fig. 10, Plate II, represents likewise a balsam preparation.

(*f*) An altogether superior diatom test-object, which can be perfectly resolved only by means of the most powerful systems, is the *Surirella gemma* Ehrbg. It should be mounted dry, and even in that condition it is extraordinarily difficult to resolve. Plate II, Figs. 11–13, represents a view of it magnified by objective-systems 500, 700, and 1200 times. The form of the *Surirella* is oval and the ends are but a little pointed. The oval surface is marked over with a framework of strong siliceous

bars which run across, at one side are quite irregularly joined to the thickened border, and on the other to the longitudinal middle line. In the fields between these bars not the least trace of markings is discernible, even with a magnification of 500 diameters. See Fig. 11. With an objective magnification of nearly 700, lines become visible in the fields which run parallel with the cross bars. See Fig. 12. Indistinctly with this magnification, but clearly with one of 1200 to 1500 diameters, these lines appear to consist of small dots. The whole field makes the impression as if it were filled with a basket-like tissue. See Fig. 13. This, according to Dippel,[47] gives ground for the supposition that over the continuous cross lines which are relatively strong, run very delicately drawn longitudinal lines, the latter being seen only, for the most part, with oblique illumination. "These diatoms give a right beautiful image when the latter method of illumination is used, and when the rays touch the longitudinal *striæ* at about an angle of 25° to 30°."

We herewith conclude the series of objects which serve us as the best tests for the optical powers of the microscope. The most important of these are, however, the scales of the *Hipparchia Janira* and the frustules of *Pleurosigma angulatum*. To these therefore we have devoted the most particular description. Still we might here add a remark as to test-objects in general. Many dealers furnish test objects with cover-glasses 0.15 to 0.20 mm. thick. We esteem these of no practical use. For if we undertake to use one of them with very high powers, we shall find the cover-glass so thick as to prevent our focussing down to the object itself, and so in spite of the correction screw the image would be ruined. We should follow the lead of Möller, and mount test objects under a cover-glass not more than 0.05 to 0.08 mm. thick.[48]

To Leopold Dippel belongs the credit of having exactly determined the direct distance between these transverse or longitudinal markings upon the scales of the butterflies' wings, the diatoms, etc.

In furtherance of our present aim we give in the annexed

[47] Dippel, *l. c.*, p. 131.

[48] Möller in Wedel, Holstein, offers such at the price of 1.50 Marks.

table the results arrived at by this well-known naturalist.[49] (Cross markings are always meant except in *Surirella* where longitudinal lines are intended. In *Lycaena*, *a* signifies a bright colored and *b* a dark colored scale.)

Name of Test Object.	Manner of Mounting.	Number of *striæ* to the 0.01 mm.	Distance apart of the *striæ* in Millimeters.
Pinnularia nobilis Ehrbg	Balsam	4 — 6	0.00208
Pinnularia viridis Rbh	"	7 — 8	0.00153
Hipparchia Janira F.	Dry	10 — 12	0.00099
Lycaena Alexis F. *a*	"	10 — 11	0.00096
" " *b*	"	14 — 15	0.00074
Pleurosigma balticum Sm	Balsam	14 — 15	0.00074
Pleurosigma angulatum Sm	Dry	22 — 23	0.00046
Grammatophora marina Sm	Balsam	25	0.00041
Nitzschia linearis Sm	"	28 — 29	0.00036
Surirella gemma Ehrbg	Dry	30 — 32	0.00032
Grammatophora oceanica Ehrbg	Balsam	32 — 34	0.00031

Applying the tests in the series in accordance with the arrangement in this table, one can come to a clear understanding of the excellency and of the working qualities of his instrument.

In conclusion it may be mentioned that Nobert for a long time has been making and furnishing an apparatus for testing the microscope without the use of test objects. It is called "Nobert's test plate" (Probeplatte). It consists of a number of groups of very delicate lines which are cut in glass by means of a diamond, or eaten into it by means of hydrofluoric acid. The lines of the several groups are at different distances apart, as for example, those of the first are 0.002256 mm. and of the last group, 0.000282 mm. asunder. The distance apart of the lines of the groups lying between these, gradually pass in value from that of the former to that of the latter. It is evident that with the help of this plate one may very easily determine the resolving power of a system, if he will begin with the first group and work progressively through toward the more difficult till he has reached a point where his lens will no longer resolve the lines. The Nobert test plate would certainly supersede all

[49] Dippel, *l. c.*, p. 134 *f.*

other test objects, did not its very high price, made necessary by the almost incredible fineness of the work, interpose the greatest barrier to its general distribution.*

Since we have now finished the consideration of the optical parts of the microscope, we will undertake to describe the other parts, only however in respect to their principal features, the stand and the illuminating apparatus. We will first describe the microscope-tube.

VIII. THE MICROSCOPE-TUBE.

The microscope-tube is a solid tube of brass, whose length is adjusted to the construction of the optical apparatus and relatively to the coöperation of the objective and ocular. In the medium and larger microscopes, its length varies between 18 and 28 cm. Beneath, it carries the ["Society"] screw for receiving the objective-system. Within its upper opening which is made cylindrical to receive it, the ocular is set. Within the tube are placed several diaphragms for cutting off certain rays which would injure the microscopic image. The tube is blackened on the inside, at least in the lower part and up to the topmost diaphragm. [In some instruments, lining the inner tube with lusterless black cloth will improve the definition by removing a scarcely noticed glare of light reflected from the imperfectly deadened surface of the brass tube. In other stands, diffused light enters the short oculars from the adjacent surface of the tube that has been brightened by contact with the longer oculars: this is prevented, by some makers, by so shaping the oculars or so guarding the inside of the tube that all oculars, whatever their length, will come into contact with the tube for exactly the same distance. R. H. W.]

The larger microscopes commonly have draw-tubes also. The tube then consists of two parts, one shoving into the other like a telescope, so that it may be drawn out to different lengths, according to need. On the advantage of this arrangement, Harting has remarked as follows:[50]

[50] Harting, Mikr., page 157 *f.*

*On account of the recent death of Nobert, his rulings will become increasingly scarce; but plates approaching them in quality, and serving the same purpose as tests, are now ruled at a far less cost by C. Fasoldt of Albany, N.Y., and by other makers.—R. H. W.

"In a microscope to which belong several oculars and objectives, it would be unreasonable to expect that one and the same length of tube would be best for all combinations. An examination previously made will serve to show at what length of tube the various optical parts will do their best work, and this can be noted and followed in the future."

"A second advantage of this contrivance consists in this, that by the inward and outward movement of the inner tube, the magnification can be brought to any definite number. For making micrometric measurements this is very important. It is simpler for instance to have the diameter of the image divided by 500 than by 487 or 513, or by 100 than by 93 or 107. Also in many observations, it is important to have the field of view of a certain definite size, 1, 2, 3, etc., mm. But this can be brought about only by increasing or diminishing the distance between the ocular and the objective, and this may be done without damage to the image if certain limits be not overstepped."

"For this purpose the inner tube should be graduated. The optician, or the owner of the microscope, can then, by careful investigation, construct a table which shall indicate the point on the graduation which will be of service in actual work with various combinations and magnifications."

[A third use of the draw-tube is, by being pushed in, to reduce the length of the body to much less than its usual standard, for the purpose of adapting it to the vertical position often required in laboratory work. To this end, such stands as are most suitable for histological work are made with a very short body, about 12 cm. long, which may be increased to the ordinary length when using the instrument in an inclined position, by extending the draw-tube as represented in Plate III. In Plate X the draw-tube is wholly closed. The stand shown in Plate XI has two draw-tubes, one within the other, to give greater range of length. By this extreme shortening of the body, the ocular is brought as near as possible to the table and one is enabled, with a minimum of discomfort and fatigue, to lean over the stand and look down the vertical tube. While the optical corrections are visibly disturbed by great shortening of the tube, there are many objectives of moderate capacity whose performance is not materially

injured, and others whose screw collar adjustment is capable of fairly correcting the evil produced.]

[The draw-tube usually is, and should in all cases be, provided at its lower end with an adapter having the society screw for the reception, when required, of an analyzing prism, a spectroscopic arrangement or an objective to serve as an erector. An objective, having too great focal length to be employed in the usual manner, may likewise be inserted here and, by sliding the draw-tube, it may be focussed through the empty nose-piece upon the object below it. Should the screw become bright from use and reflect false light, it must be guarded by a ring of hard rubber or blackened brass screwed into it. This will not only render it harmless but will become an efficient diaphragm to stop stray light from other sources. R. H. W.]

The microscope-tube is moved by a propelling mechanism, rack and pinion, or by free hand. In the latter case it is necessary that the tube should exactly fit into the inclosing sheath. This is secured by careful polishing of both surfaces or by lining the inside of the sheath with cloth [or by the use of springs]. The tube should never be oiled, but it and the sheath kept always absolutely clean.

When we are examining one object after another with different magnifying powers, it is necessary in each case to screw another system into the tube.

[The screw by which the objectives are attached to the tube is of standard size, devised and prepared, in 1857, by the London (now Royal) Microscopical Society, and therefore known as the "Society" screw. Being, practically, in universal use in both this country and England, and even applied to continental objectives intended for sale here, this standard screw renders objectives of the different makers interchangeable, so that a student may use his set of objectives upon a variety of stands, or may purchase any desired lens without doubt as to its harmonizing with his former apparatus.]

[The introduction of the society screw, with all its attendant advantages, was a loss in respect to ease of manipulation, as it put a stop, temporarily, to the use of bayonet catches and other devices designed to lessen the labor and delay occasioned by frequent changes of objectives while the instrument was in use.]

[IX. NOSE-PIECES.]

[Subsequently double, and even triple and quadruple nose-pieces were used, the upper portion of the apparatus being attached by a society screw to the compound body, while the lower portion or revolving plate carried the specified number of objectives permanently screwed into it, so that any one of them could be rotated into use, in the axis of the instrument. Such a triple nose-piece is shown in Fig. 18. When well made, very light, and of the angular form, this device is very satisfactory in use, and greatly relieves the labor of investigations requiring frequent changes of power. It is however somewhat costly and cumbersome, and its weight, including that of the attached objectives, impairs the delicacy of the fine adjustment, especially in some of its older forms.]

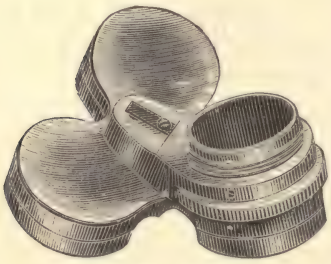


FIG. 18.

[During the past two or three years there has come into existence a new series of contrivances, by which objectives though attached one at a time can be changed with ease. In them the nose-piece is a single chuck, permanently screwed into the microscope body, some mechanism being included by which the objective can be instantly seized or released. No change is made in the screw of the microscope-tube or of the objective, and the collar screwed upon the objective should be of such size as to be left in position when the objective is packed in its box. These nose-pieces require excellent workmanship, in order to secure accurate centering and adequate stability, and in their use care should be exercised, especially by inexpert hands during the manipulations required for the screw-collar adjustment of the objective, not to unintentionally release the objective from the grasp of the chuck. Should experience give weight to this drawback, slight modifications of the apparatus would doubtless remove the difficulty.*]

*Constant use of the "Facility" nose-piece for more than a year does not reveal the existence of this possible objection.—A. B. H.

[Such a contrivance is the "Facility" nose-piece, Fig. 19, which is simply a self-centering chuck, which seizes the objective by a small ring or collar permanently screwed upon its society screw. It was devised and is made by James L. Pease of Chicopee, Mass. Of the same general character is the "Congress" nose-piece contrived by Prof. Albert McCalla and made by W. H. Bulloch of Chicago, Fig. 20, in which a chuck, with three slots, grasps, by three projections upon it, a ring permanently screwed upon the objective. The contrivance still more recently produced by Mr. Zentmayer, Fig. 21, is one in which a nose-piece is screwed into the microscope-tube and a collar permanently screwed upon the objective, the collar and nose-piece being connected by a screw, the opposite quarters of whose threads are cut away, so that insertion can be accomplished without screwing and the inserted collar locked fast by a single quarter-turn which will bring the screw-threads of the two pieces into relation with each other. An index-mark upon the nose and a corresponding mark upon each collar indicate the position in which the collar can be inserted; and a jam-nut enables the nose to be set in such position as to bring its index in front of the microscope or in any location preferred by the observer. This contrivance has been successfully used in the arts, as in the construction of breech-loading cannon and in the coupling of hose for use

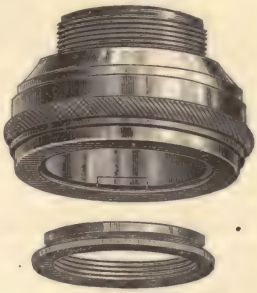


FIG. 19.

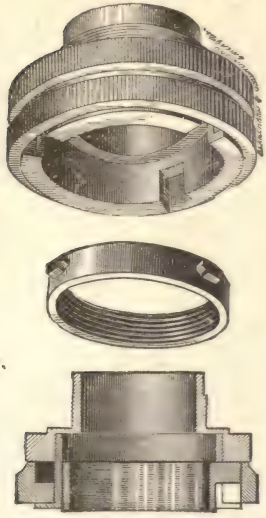


FIG. 20.

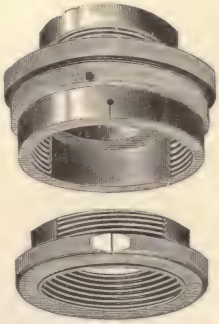


FIG. 21.

with "fire-engines." It was proposed by Mr. E. M. Nelson at the Queckett Club, Sept., 1882, to similarly cut away portions of the society-screw from objectives and stands, as a means of instantaneous attachment. No general effort has been made, however, to introduce it for this purpose, and it is doubtful if the modification could be effected by the various makers with such uniformity that their work would be really interchangeable.]

[The latest contrivance of this sort is by Mr. Charles Fasoldt of Albany, N. Y., who makes a nose-piece the alternate sixths of which have the thread cut away, while one of the remaining sixths is movable and capable of being withdrawn from contact by a lever reacting against a strong spring, as shown in Fig. 22. The objective requires no preparation, and can, after starting by pushing back the movable section of the screw, be screwed in in the usual manner. If, however, the lever be pressed down with the thumb, the movable section of the screw is withdrawn so far that the objective can be placed at once in position against the shoulder above. Releasing the lever allows the movable portion of the screw to slide firmly back into place and grasp the threads of the objective, which is held exactly as if it had been screwed in, and which can be tightened up against the shoulder, if necessary, by a single screwing movement. A jam-nut is provided, as in the Zentmayer form, by which the lever and index can be set in a position most convenient to the observer. A corresponding mark should be made upon the brass mounting of every objective to be used, the mark upon the objective corresponding to the index of the nose-piece, not when the objective is snugly screwed up to the proper tension, but after it has been unscrewed about one-eighth of a revolution. When then inserted, in this slightly unscrewed position, the threads are grasped with more certainty and effect, and a slight twist, one-

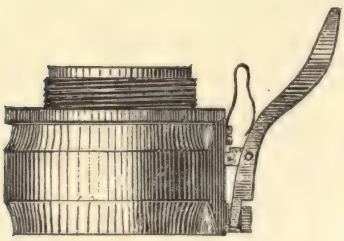


FIG. 22.

eighth turn to the left, sets it with uniform and unerring firmness against the shoulder. After experience renders this twist habitual, it becomes almost automatic, and is scarcely distinguished as a part of the apparently single action of putting the objective where it is wanted. When in position, the objective cannot fall out or be pulled out, without being either unscrewed or else instantly released by pressing down the lever that opens the screw. R. H. W.]

X. THE FINE-ADJUSTMENT.

The fine adjustment screw, as we have pointed out, is a contrivance by which, when the coarse adjustment has brought the optical apparatus into the immediate neighborhood of the object, it can be adjusted with almost mathematical exactness, so as to bring the object exactly into the focal point of the lens. How this adjustment was brought about in the older microscopes we have already mentioned, p. 7. With smaller microscopes, to this day, the fine adjustment is occasionally found on the stage. This is objectionable on instruments used for scientific observation. In microscopes designed for scientific investigation the fine-adjustment screw is placed in the perpendicular pillar [or variously shaped limb] which bears the tube.

[Until a very few years ago, the fine adjustment of the best English and American stands consisted of a light tube or nosepiece, sliding easily within the main body of the microscope and projecting slightly below its lower end; this tube being pressed firmly downward by a spiral spring, and being raised by a lever, actuated by a screw which was turned by the thumb and fingers of the observer's hand. The position of the lever and screw varied with the caprice of the maker, but it was most frequently attached to the body in front and near the lower end. The objective screwed into this sliding tube could be moved up and down until its distance from the object was adjusted with great precision. The arrangement, however, lacked firmness, especially in handling the screw-collar adjust-

ment, and it often became much the worse for wear. Some persons, also, feared that the slight change it effected in the distance between the objective and the ocular might be practically as well as theoretically objectionable.]

[Meanwhile Continental microscopes were made with a fine adjustment moving the whole body of the instrument. In most of those which found their way to this country or were imitated here, a hollow pillar was provided, within which was contained a solid cylinder firmly attached below to the foot, or to the movable portion of the trunnion-joint. The outer portion (a hollow tube sliding over the inner) carried with it by means of a transverse bar the whole body of the microscope, the vertical movement being accomplished by a screw working against a spring; just as if, in Plate XI, the milled screw-head, high up at the right of the plate, were made (which it is not) to carry up and down the outer and visible portion of the column below it over an inner and concealed column. This form of adjustment lacked smoothness and uniformity of movement, was particularly subject to side motion, and had no good means of taking up the loss from wear. Its use in this country was very limited and mostly confined to stands of low grade.]

[In 1876, among the novelties prepared for display at the Centennial exhibition, Mr. Zentmayer transferred the fine adjustment of his stand from the nose-piece to the limb, making the bar just behind the body slide upon plane surfaces, as in the coarse adjustment, a sufficiently delicate movement being imparted to it by a lever acted upon by a screw at the left. Great steadiness and indefinite capacity for wear are attained in this way. This adjustment is shown in Plate III, the black line just back of the body and parallel with it representing the edge of the sliding surfaces. In Plate IX this is shown combined with a rack and pinion coarse adjustment, a separate sliding movement being provided for each.]

[In Mr. Bulloch's microscopes, Plate X, a similarly situated screw and lever give a very delicate vertical motion to a sliding box, in which is set the pinion of the coarse adjustment itself as a portion of the fine adjustment.]

[In the Bausch and Lomb instruments may be found the so-

called "clock-spring" fine adjustment, shown in Plates IV and XI, and in section in Fig. 23. The transverse bar of the stand, extending from the pillar to the body, consists of a hollow box whose top is represented by *d*, whose front, towards the right, is open, and whose back, towards the left, is either attached to the pillar *c* by screws, or cast in one piece with it, as in Plate IV. From the back of this box, and firmly attached to it, project forward two stiff horizontal steel springs *aa*, which bear at the right the plate *e*, containing the pinion *f* of the coarse adjustment *fg*. An arm of *e* projects backwards and is pressed down by the carefully cut fine-adjustment screw *b*, reaction being furnished by the springs *aa*. The horizontal and vertical portions of *e* being inflexible and continuous, and the pinion-rack and body, *f* to *h*, having no other support than the springs *aa*, it is evident that any vertical movement of the screw *b* must impart a like movement to the body *h* and to the optical parts contained therein. Since *a* and *a* are parallel and of equal length, the motion of the body *h* must always be in a direction absolutely parallel to the pillar *c* and vertical to the plane of the stage. The theoretical movement of *h* to and from *c* is so slight as to be unnoticed. This form of adjustment is free from lateral motion or lost motion, has no friction except that of the screw itself and does not deteriorate by age or wear. A somewhat similar system, though not exempt from friction and wear, is employed in the stands of Seibert and Krafft described by Dr. Behrens, in which a par-

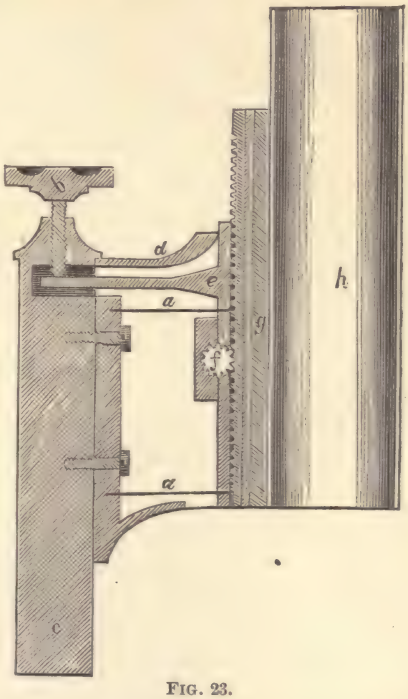


FIG. 23.

allel motion is secured by a system of levers instead of springs. R. H. W.]

XI. THE STAGE.

The object-table, or, for short, the stage, is a solidly wrought metal plate which, in respect to the optical apparatus, assumes an unalterable position. The optical axis of the microscope must be exactly perpendicular to the plane of the stage. The form of the stage as in Plate X is rectangular, or, as in Plate XI, round. Both forms are alike practical, assuming that they are roomy enough to be convenient to handle. Small stages are objectionable. They should at least be large enough so that the largest form of slide would not reach from side to side. In order to obviate the reflection of rays from the stage it is commonly blackened [at least on its lower side].

Large microscopes are usually furnished with rotating stages, and this arrangement is very convenient, as it enables one to turn the object quite about upon its axis without being obliged to disturb the slide. And besides, when the rotating stage is graduated and made to work by an index, it can be used with good results in measuring the angles of crystals. In the latter case, the stage should be provided with a screw arrangement by which it may be exactly centered, so as to bring that part of the object to be examined into the exact optical axis of the microscope. In the illustrations, Plates X and XI, such rotating stages are represented [centering adjustment being applicable if specially ordered]. The circular plate can be set in rotary motion upon the stationary under-piece. It has a milled edge so as to be moved more easily by hand.

[Many of the small and cheap microscopes are now made with a plain round stage, a form unobjectionable in itself and capable of a higher development than the square. The stage, for instance, in Plates IV, V or VI, is of extreme simplicity, but is capable, by reason of its circular form and location (concentric with the optical axis), of receiving, either originally or subsequently, such an upper plate as those shown in Plates X and XI, thus making a simple but serviceable revolving stage.

Such an arrangement, besides its other good qualities, is compatible with that extreme thinness of stage which is now considered essential in order that freedom of illumination be not interfered with. R. H. W.]

It is sometimes necessary to make the object fast to the stage in order to devote a considerable time to the examination of a single point, or in order to be able to turn the microscope down to an oblique position. For this purpose a simple clamp arrangement is sufficient.

[The commonest form, and one answering, in skilful hands, nearly all useful purposes, is a pair of spring clips of steel or brass attached to the stage by screws or pins, as shown in Plates III and XI. Beneath these the object-slide is placed and can be successfully manipulated, even under moderately high powers. A bar of glass or brass, sliding under short clips, forms a sufficient ledge for the support of a trough or receptacle too large to be placed under the clips. A somewhat more delicate adjustment can be obtained by using a brass object-carrier sliding over a glass stage as in Fig. 24, which is designed as an addition to the stand shown in Plate IV; or a glass object-carrier, or sliding stage, which is especially adapted to chemical work and is easily applied to any stage of the model shown in Plate IX.]

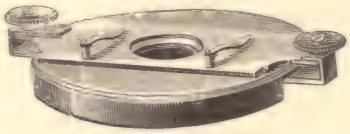


FIG. 24.

[While elaborate "mechanical stages," on which the object can be moved in every direction by racks and screws, are not required for general use and are much less esteemed and used than formerly, still there are some procedures, such as micrometry and the use of extremely high powers, where a stage having some sort of mechanical movement is a material advantage. Such stages are now made in simplified forms, thin enough to conform to the present demand for thin stages, and small enough to be applicable to the smallest stands described in this book. R. H. W.]

An arrangement seldom employed by the botanist, but more frequently used by the zoölogist, is a warming stage. By means of it, the preparation may be kept at a higher temperature than

that of the surrounding atmosphere. Max Schulze[51] has invented such a stage capable of being heated. It consists of a metal plate which is fastened to the stage by clamps. Corresponding to the opening in the stage there is a place bored out in it for illumination. It carries in front, in the middle a diagonally placed thermometer and two long arms extend out far beyond the stage; under these are placed two spirit lamps as heaters. The lower end of the thermometer is wound about the opening on the plate and so gives the exact temperature of the object.

XII. THE ILLUMINATING APPARATUS.

The illuminating apparatus consists essentially of three instruments, viz.: the mirror, the diaphragm and the condensing lens. The mirror is placed beneath the stage, but the diaphragm is placed near, if not upon the under side of the stage itself.

A. The Mirror.

The mirror is formed of a circular metal frame of 30 to 50 mm. [or more] broad, in which is mounted, on one side, commonly, a plane glass mirror, and on the other, a concave mirror of spherical form. [The mirror is fastened to the arm, as seen in Plates III and XI.] The mirror frame is mounted upon an arm so as to revolve upon it and the arm is so constructed as to enable the mirror to be moved right and left, and placed in any position with reference to the opening in the stage.

It is by no means a matter of indifference, for the illumination of the object, which of the two mirrors, the plane or the concave, is used, as will be clear from what follows. We will suppose that there is a source of light *l*, Fig. 25, which sends rays upon the mirror *s*, as shown in the illustration, *la*, *lb*, *lc*, *ld*, *le*. It is known that the concave mirror converges the rays that it reflects. It will be seen without further elucidation that, by giving the mirror its proper position and distance from the

[51] Frey, Das Mikroskop, page 65.

object, all the rays which fall upon it will be concentrated upon the object that lies at *p*, upon the stage between the slide and the cover-glass. In this case very many rays are concentrated upon a very small space, the object, which must consequently be very brightly illuminated. The parallel rays of daylight, which are commonly used in microscopical investigations, behave, when they fall upon the concave mirror quite like the diverging rays we have just been considering.

The plane mirror works very differently. The parallel rays falling upon that are reflected at the same angle, consequently run parallel on their way to the object. If we now suppose

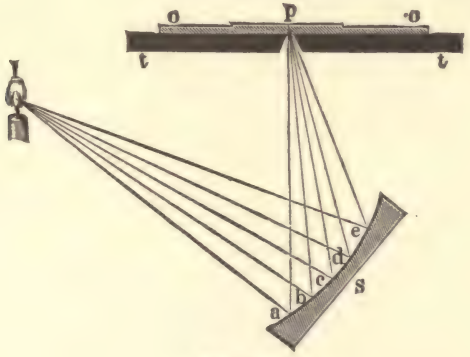


FIG. 25.

that there falls upon the surface of the mirror *n* rays of light and the stage diaphragm has but $\frac{1}{10}$ the extent of the mirror surface, it follows that the plane mirror will afford but $\frac{n}{10}$ of the light, to the object, that it receives, while under the same conditions, the concave mirror would contribute to the illumination of the object, all of the rays that it receives. Still less of the illumination will be given to the object when it is received from a near source of light as in Fig. 25, for then the rays are diverging, and on falling upon the plane surface, will be still more diverged, and thus spread more widely, and, so to say, thinly, over a given space.

For obvious reasons, therefore, the plane mirror should be used with low powers, and the concave with higher and the

highest powers. From the above considerations also, it is easily seen why the plane mirror affords, on the whole, stronger lines of definition than the concave. Very delicate structures, which are to be observed with low powers, appear with much sharper outlines when illuminated by the plane than by the concave mirror. For many objects, with medium or high magnification, illumination must be had from the concave mirror with oblique light, in order to be able to recognize certain fine details. This oblique illumination is produced by moving the mirror to a certain angle right or left, and adjusting the diaphragm so that the cone of rays may still reach the object on the stage. Now the outlines will give broader shadows than before and so will be much more easily recognized. The different effects of central and oblique illumination become clear by using first one and then the other illumination upon the diatoms, as, for example, *Pleurosigma angulatum*, *Pl. formosum* or *Pl. balticum*.

B. Diaphragms.

Only in rare cases will one be able to obtain the desired illumination by means of the mirror alone. In many cases it is important to shut off from the microscopic image the border rays and even the central rays that are reflected from the mirror. Both can be accomplished by means of the diaphragm attached to the stage.

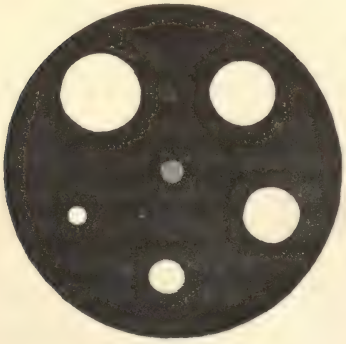


Fig. 26.

1. *The Revolving Diaphragm.* In the older instruments and in the smaller ones of the present time the marginal rays are shut off by means of the revolving diaphragm, Fig. 26. It is a plate of metal, not too thick, blackened on both sides and provided with several round holes of different diameters, but whose middle points are at the same distance from the center of the disk. The diaphragm is fastened to the under side of the stage [or to some supporting apparatus

beneath the stage] by a screw passing through its center, and about which it is made to turn by the fingers, its openings being brought successively under the opening in the center of the stage.

But it is difficult faultlessly to center this diaphragm, and what is a worse evil it is not on the same plane with the top of the stage but with the under surface, and the consequence is that between the diaphragm and the object a cone of dispersing rays is formed which very considerably injures the clearness of the microscopic image. To avoid this fault the diaphragm is sometimes made in the form of a concave segment of a globe, and the under side of the stage is hollowed out to correspond. But this contrivance allows no very near approach to the object, and the difficulty of correct centering is still greater, and the objection to this kind of diaphragm increases with the power of the objective employed in the investigation.

[In a few American stands, as in some of those made by Mr. Grunow of New York, and in one of Mr. Zentmayer's, the revolving diaphragm plate is, for this reason, let into the upper surface of the stage, so that when in use it lies almost in contact with the object-slide. In most American microscopes, however, it is attached to the under surface of the stage or fixed at a moderate distance below it, in order to secure that control of the angular breadth of the illuminating pencil, which is obtained by locating the diaphragm at a sensible distance below the point of convergence, *p*, in Fig. 25, of the cone of rays condensed upon the object by the concave mirror. By far the best arrangement is to have the diaphragm-plate so mounted, whether in a sliding-tube or upon a sub-stage, that it can be set at any level from the plane of the top of the stage to a plane five or ten mm. below it, as in Plates IX to XI. R. H. W.]

2. *The Cylindrical Diaphragm* is a contrivance which quite obviates the evils mentioned, as belonging to the disk diaphragm, and it is so simple and so well adapted to its purpose that it has quite driven the others out at the present time. It consists of a hollow cylinder exactly turned without and blackened within. The upper end is drawn suddenly in to form a raised ledge of somewhat less diameter than the opening in

the stage. Over this upper edge of the cylinder are placed small metal caps which are provided with central openings of various sizes.

[This excellent piece of apparatus, which has not yet come into very general use in this country, is a survival of the "dark well" or "dark chamber" of the early days of the modern microscope. It may be slipped from below into a ring or short tube like that projecting from the lower surface of the stage in Plate V, or supported by the sub-stage, as in Plate X, or in microscopes of very simple construction inserted into a special carrier to be presently described.]

3. [*The Iris Diaphragm* is, by far, the most perfect means of limiting the cone of illuminating rays, and is now produced in various forms by numerous makers. That of the Bausch and Lomb Optical Co. is represented in Fig. 27. It consists of a dark well, closed at the top by a series of thin movable plates, which by a very simple mechanism may be made to close the opening altogether, or to open gradually and form a practically circular aperture of any desired size up to the maximum capacity of the well, the size of the opening being controlled by the milled head at the bottom. Such ease and precision are secured by this contrivance, in adjusting the amount of light admitted, while the object is under observation and all the adjustments of the stand and light remain undisturbed, that a person once accustomed to its use is little likely to be satisfied without it. How it may be combined with the condensing lens will appear further on. R. H. W.]

FIG. 27.

4. [*Special Diaphragm-stops.*] All the contrivances thus far considered are adapted to shut off the marginal rays which the mirror reflects upon the object. Latterly, attention has been given to correct the central rays which proceed from the mirror. It has been found that they frequently injure the microscopic image; particularly so is this true in the use of a condenser, where one must often provide for their elimination. This can be done very simply by the use of a "central-stop." A central-stop is a small circular metal plate which is supported

on the slenderest possible metallic arm [or on a thin glass disk], and which by some contrivance may be brought into the middle of the condensing lens.

[A horizontal slit, consisting of a central opening much longer than wide, in the diaphragm plate, is used to great advantage in connection with the condensing-lens, in the illumination of binocular microscopes, sufficient angular breadth of illumination being thus secured to light both fields of the instrument freely without such an excess of light as would impair the view of the object. A corresponding effect is attained by the use of a pair of horizontally arranged circular apertures separated at such an angular distance apart that each one will admit the pencil of light required by one tube of the instrument.* The pair of apertures requires to be adjusted with more skill than the horizontal slit, and apparently without compensating advantages. With these stops, shown in Fig. 28, the use of the binocular may be extended to higher powers and angles than without them. Special stops for oblique light or other effects may be used at the option of the student. Any of the special stops may be cut in certain portions of the revolving diaphragm-plate or in some of the caps of the cylindrical diaphragm which must be substituted for the iris diaphragm during the time of their use. R. H. W.]

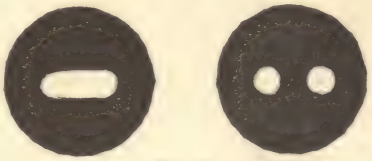


FIG. 28.

C. CONDENSERS.

For certain purposes it is recommended to interpose a lens between the mirror and the object which will concentrate the rays of light from the mirror exactly on one point. This can be done best with a condensing lens of very short focus. We commonly use a plano-convex with the convex side strongly curved.

[Such a condensing lens is often required, not only to in-

*See a figure and description of this method of binocular illumination by the writer in the American Naturalist of December, 1870, p. 636.

crease the amount of light reaching the object, but also to secure effects dependent upon the obliquity with which the light passes through it. The simplest arrangement and one of the best is a nearly hemispherical lens, 10 to 12 mm. in diameter, stuck to the bottom of the object-slide, directly beneath the object, by a minute quantity of glycerine or oil of cloves. The lens should be less than a hemisphere by about the average thickness of an object-slide, so that when the two are united the object will be at the center of curvature. Light, either parallel from the plane mirror or condensed by the concave mirror, may then be passed with a peculiarly brilliant effect, directly to the object from the mirror in whatever position, from axial to the level of the bottom of the stage, in which the mirror may be placed. If the obliquity chosen be in excess of the semi-aperture of the objective, light will pass through the object but not directly into the objective; the object, if neither too opaque nor too translucent, then appearing brilliantly illuminated upon a dark field, the same effect being produced with less intensity by the prism mentioned below, or with very limited brightness and only for very low powers, by the concave mirror alone in a very oblique position. The condensing power of the hemisphere is small, on account of its large curvature and the position of the object far within its focus. If greater refraction be desired, the "Wenham button" may be substituted, whose sharp curvature and more precise focus give a more intense illumination, but one adequate only for minute objects and applicable chiefly to the higher powers. For illumination with parallel instead of converging rays a small triangular prism may be similarly attached to the slide, the effects being the same except that the light is not condensed and that its obliquity is limited to one angle, or if the prism be revolving and not equilateral, to two or three angles. Such a prism upon a convenient mounting is shown in Fig. 29. It is of far less general applicability than the hemispherical lens. Both lens and prism are somewhat difficult to locate exactly in the required position, and are liable to slip out of place if the stand be inclined, especially if too much of the connecting liquid be employed. For these reasons they should be mounted, for stands having a sub-stage, at

the summit of a vertical wire rising from the center of the sub-stage. When there is no sub-stage, this wire may be supported by an arm attached to the stage itself, as in the ingenious device of Jas. W. Queen & Co., which appears, with prism attached, in Fig. 29.]

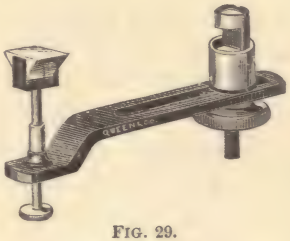


FIG. 29.

[If the lens be mounted at the top of a dark well, its angular capacity will of course be limited by the position of the lower edge of the tube which should, for this reason, be short and broad. It can then, however, be slipped away from the object, downwards, its glycerine contact being omitted, and focussed upon the object from below. In this case its capacity as a condenser is greatly increased by placing beneath it a still larger lens called a collecting lens. Condensers of two large non-achromatic lenses have been extensively used with great success for many years, largely through the influence of Dr. Beale in advocating the use of an ocular for that purpose; and, notwithstanding recent improvements in this direction, one may still with much satisfaction transfer his highest power ocular to a ring beneath the stage, as a condenser. In the orthoscopic ocular, similarly used, and the "Webster" condenser, an achromatic upper lens is employed; while in the latest and now most approved form, introduced by Professor Abbe and hence called the Abbe condenser, both of the lenses are non-achromatic and of such great thickness that the top of the lens will nearly touch the object-slide when focussed upon the object. By this arrangement not only is great aperture (n. a. 1.42 and upwards) readily obtained for use with the highest-angled objectives, but water or glycerine contact with the object-slide becomes practicable, giving an "immersion" illuminator with increased working capacity. This simple and inexpensive combination seems to be superseding, with good reason, all the elaborate and carefully corrected achromatic condensers formerly used.]

[By combining a black center-stop with a condensing lens or system, a central cone of light below the object and a corre-

sponding inverted cone of light above the object will be suppressed, each having the same angular aperture as the obstructed portion of the condenser. If an objective of less than this aperture be used, it will of course receive no direct light, but will view the object illuminated by the oblique rays from the unobstructed marginal position of the condenser, the field meanwhile remaining dark. For low powers exclusively, a single thick lens with a central black stop attached to its upper surface, known as the spot lens, is sufficient. An achromatic condenser with center-stops, or the Wenham paraboloid, a truncated glass paraboloid, with a center-stop, to give by internal reflection a hollow cone of rays condensed at a large angle, has been heretofore employed with the higher powers; but the large lens-systems of the different varieties of Abbe condensers, with their large apertures and immense amount of light, if provided with suitable center-stops, leave little to be desired, with either high powers or low. Objectives of too large aperture for this method of illumination are frequently brought within its scope by inserting a diaphragm behind them to temporarily reduce their aperture to a practicable limit. This so-called black ground or dark field illumination is very effective with many delicate vegetable hairs, fibres, etc., which should usually be viewed dry or in water, as balsam renders them too transparent to arrest and disperse sufficient light. R. H. W.]

[D. Illuminating Combinations.]

[1. *The Universal Accessory.* For the sake of convenience, some of the opticians combine into one piece of apparatus, to be fitted below the stage, several of the sub-stage appliances, such as diaphragms, condensing lenses, polarizing prism, etc. A simple arrangement for this purpose, being inexpensive and applicable to smaller stands, is the "Universal Accessory" of Bausch and Lomb, shown in Fig. 30. It consists of a rather thick stage-plate intended to lie upon the stage in place of the object slide, and to carry the slide under a pair of spring clips upon its upper surface. Set into the centre of this plate and projecting below it through the central opening of the stage, is a short

revolving tube to receive a cylindrical diaphragm, polarizing prism, or condensing lens; the latter becoming, with a black centre stop, an efficient spot lens. This apparatus is especially suited to stands having no sub-stage conveniences.]

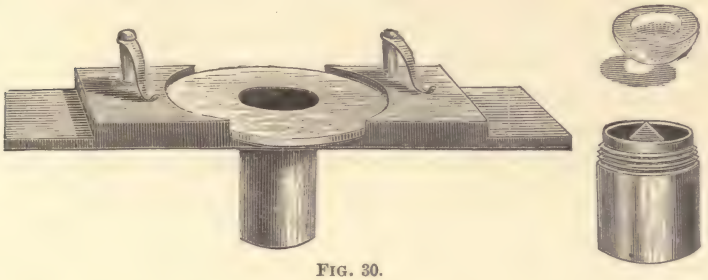


FIG. 30.

[2. *Ward's Iris Illuminator.* A more elaborate and effective arrangement, constituting an illuminator suitable for work of a higher grade, is a combination of the condensing lens with a decentering iris diaphragm, devised by the writer and made by Bausch and Lomb. It is shown in Fig. 31, and consists of any

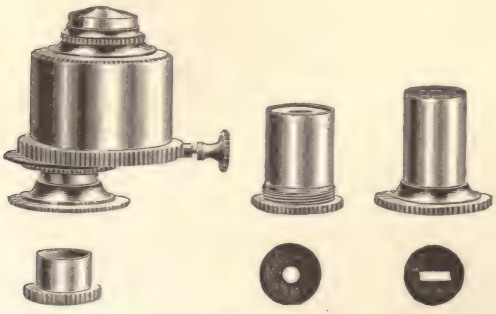


FIG. 31.

desired lens system, either "dry" or "immersion," under and close to which is mounted an iris diaphragm set in a sliding plate so that it can be moved into any position from the center to the periphery of the system, without altering the position of the latter. Thus not only the obliquity of the light, but the

exact amount desired or found advantageous at any chosen obliquity can be regulated with perfect precision by a touch of the hand to the decentering screw and to the adjusting collar of the diaphragm. This contrivance can be applied, without outgrowing the limits of the customary 1½ inch (38 mm.) sub-stage tube (which size of sub-stage is being very generally adopted, and it is hoped will soon be made "standard"), to any condensing system whose posterior diameter does not exceed 21 mm. ($\frac{13}{16}$ in.). It is well adapted to a simple hemispherical lens, a large-lens $\frac{4}{10}$ achromatic condenser, or the doublet of thick non-achromatic lenses adopted by Prof. E. Abbe of Jena. In using the first or the last of these three, which have nearly superseded the late "achromatic condensers," it should not be forgotten that the best performance is nearly always obtained by connecting the illuminating lens with the object-slide by a drop of water. A blue glass disk, for correcting the glare and color of artificial light, is fitted to a tube that can be inserted into the bottom of the dark well of the diaphragm. A special adapter is also provided for the use, in place of the iris diaphragm, of central stops for securing dark field illumination; and a revolving tube, slipping inside of this, carries a horizontal slit, or pair of horizontally arranged apertures, for the better illumination of binocular microscopes (see page 40), or special stops for the production of any effect desired by the user. In similar fittings, may be mounted a polarizing prism and selenite plate, a small Nicol's prism being sufficient, in connection with the condenser, to give adequate illumination for moderately high powers. The whole apparatus rotates about its own optical axis, which remains coincident with that of the microscope itself. By removing the lenses from the top of the apparatus, the iris diaphragm, with or without its blue glass disk, or the polarizer, will be found in position for use by itself. Except for very low powers, however, the illuminator may be considered as a part of the stand and kept habitually in place, the changes of light required for a great variety of work being readily accomplished by its aid. It can be applied to almost any microscope, whether with or without a sub-stage.]

E. Opaque Illuminators.

[The foregoing methods of illumination pertain to objects sufficiently transparent or translucent to be viewed by light passed through them from below. Opaque objects, viewed by light reflected from their upper surface, as frequently becomes necessary in botanical study, can seldom be adequately illuminated by the general light of the room. They usually require for satisfactory exhibition the condensation of light upon them by means of a lens or mirror. A small condensing lens may be

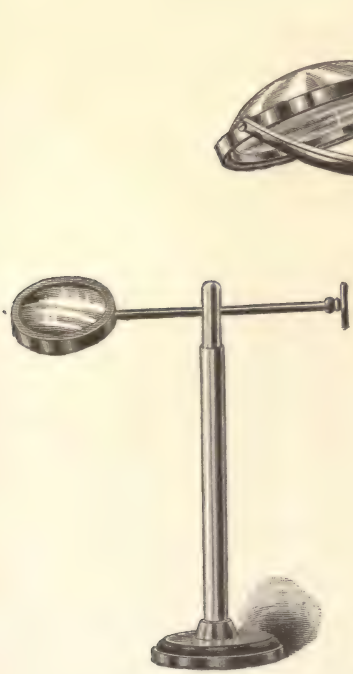


Fig. 32 *a*

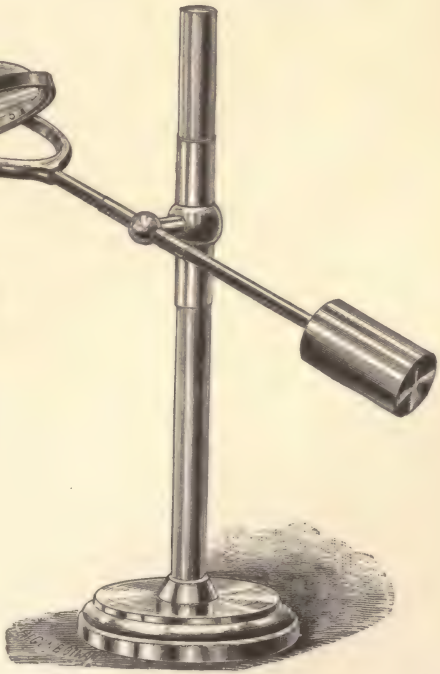


Fig. 32 *b*

attached to the microscope itself, or mounted upon a stand of its own. Large plano-convex condensing lenses called "bull's eyes," having great thickness and short focal distance, are usually mounted on a separate stand as shown in Fig. 32. They

are used near the microscope, the object being in the principal focus, to condense light upon the object, or near a lamp, the flame being in the principal focus, to give a beam of intense and nearly parallel rays for use at a convenient distance from the flame. In stands having the modern style of swinging tail-piece, as in Plates III to XI, the concave mirror can be readily brought above the stage for the concentration of light upon the object. For higher powers a small concave silvered mirror, or side reflector, is attached to the objective or to some neighboring part of the stand. Opaque objects under the highest powers can be sufficiently lighted by making the objective its own condenser, as in the case of the Beck illuminator, shown in Fig. 33, where light entering an aperture in the side of the nose-piece is reflected downward by a thin cover-glass in the center of the tube, and is condensed by the objective upon the object in its focus. In the use of such contrivances, of which there are many in use, much care and tact are necessary to avoid the glare of false light. R. H. W.]

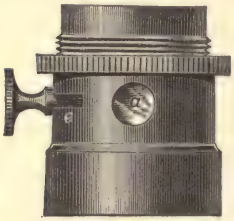


FIG. 33.

F. Observation by Artificial Illumination.

The microscope is an apparatus of the day. The best source of light for it is diffused daylight, when the heavens are evenly covered with a transparent white veil of clouds. The blue of the cloudless heavens, the changing light of hurrying clouds, and the direct sunlight are alike objectionable for careful microscopical investigations. But if one has necessary microscopical work to do on a sunny cloudless day, his best course is to take a white wall or a large white paper screen which reflects sunlight as the source of his illumination.

But it will now and then happen to the microscopist that he *must* work by the light of the lamp, it may be to make the evening hours help out the short dark winter days, or it may be to study the reproductive processes in the lower cryptogams, which take place only during the night hours. In these cases one

has to contrive some way to improve the artificial light which in and of itself is in the very highest degree unserviceable for microscopical observations. The yellow light of gas or petroleum consists, for the most part, of rays which belong to the first half of the spectrum and are, more than any other, hurtful to the eyes of the observer. Besides, if one turns the mirror directly to the flame, the field of view is commonly too bright, and if toward the lamp shade, it is too dark. In order conveniently to modify the brightness and color of the light, the following means is suggested and recommended. Direct the mirror to the brightest part of the flame and then interpose, close to the mirror, an upright screen of thin translucent paper, which one can easily make in a few moments. This subdues the brightness of the field so much as to make it painless to the eyes. In order to change the yellow color to a blue tint it is only necessary to bring between the mirror and the diaphragm, a little plate of blue cobalt glass. I use a little round glass disk, about 13 mm. in diameter and 2 mm. thick. Its color is a faint blue and the underside is slightly and uniformly ground. This little plate is placed directly under the diaphragm. In this way the illumination of the field is made uniform and soft bluish.

The following arrangement is still better and more convenient. Fill an ordinary cobbler's globe* with a pretty dark blue solution of cupric oxide of ammonia, and place it between the source of light and the mirror in such a way that the mirror can be directed toward the brightest spot of white light produced. In this way one gets an illumination for evening which leaves very little to be desired. The right concentration of the cupric oxide of ammonia is easily made out after a few trials.

XIII. THE MICROSCOPE FOOT.

Of the microscope foot two things are required. The microscope must rest on it firmly and securely. The foot should

*I am told that, in Germany, the cobblers, when at work in the evening, suspend a glass globe filled with water between themselves and the lamp, which sets upon the table before them, in such a position that it concentrates the light directly upon their work. A. B. H.

be neither too light nor too small. Formerly the microscope foot was made in a disk or plate-like form, of a thin plate of brass loaded with lead. But to this form of foot there was this objection, that, unless the surface upon which it rested was exactly flat and even, the microscope would totter or wabble with every motion. So at the present time the microscope is made with the horse-shoe foot [or with a tripod giving three widely separated points of support. The latter effect is most frequently gained by means of three solid projections on the bottom of the brass plates or arms as shown in all the Plates but especially in Nos. V, X and XI. It is now customary to give more satisfactory means of contact with the table by supplying the feet with disks of soft India rubber projecting slightly from their lower surface. R. H. W.]

The folding microscope foot, which consists of three legs, is altogether objectionable. They never give a firm support and are the most impracticable things ever devised for the microscope. At best this form is applicable only to travelling microscopes where its compendiousness is its only recommendation.

XIV. RULES FOR THE USE OF THE MICROSCOPE.

A good instrument is a very precious article, but a microscope ruined through neglect is the most hateful thing one can see. By proper treatment a microscope, though much and often used, will remain unchanged for many long years, assuming that it be not too much exposed to its mortal foe, the dust, and that it be cleaned each time after using. It may not be superfluous here to add some hints about keeping the microscope clean.

It is an easy matter to keep the metallic part clean. It is only necessary after each using to rub it carefully with chamois skin or linen. Commonly it will be sufficient to rub it dry, to restore the brightness, but sometimes water may be used especially in cleaning the stage. Alcohol and the like should under no circumstances be used on the polished parts of the microscope, because it will be sure to dissolve the lacquer with which they are covered. But its use is not necessary, since no oil or

glycerine or the like should be put on the screws or any part of the stand. The dull parts of the oculars so far as they sink into the microscope tube, the tube as far as it runs in the outer sheath, and finally the diaphragm cylinder, should be carefully cleaned.

Keeping the glasses cleaned demands still greater care. Many people believe the optical parts of the microscope are clean when no dust can be seen in the field of vision. But this is proof only that the under ocular glass, the collecting or field lens is clean. Particles of dust on the objective cannot be seen in the field of vision as a little reflection will show, but they exercise a damaging influence on the microscopic image, since they cause a cloudiness and impaired definition. It is therefore necessary to clean, occasionally, both oculars and objectives. For this purpose, old, very soft linen washed repeatedly in distilled water or a soft hair pencil with distilled water should be used.

The ocular should be cleaned in the following way. Supposing it to be quite soiled, both glasses should be unscrewed and first of all wiped with dry linen. Then dampen a clean piece of linen with distilled water and rub each glass holding it by both edges. This may be repeated as often as necessary, and when it is rubbed perfectly dry it should be swept with a fine hair pencil to remove any fibres from the linen which may be adhering to it. In this way we get perfectly clean glasses. To test the purity of a cleaned glass it should be breathed upon a little. If it is perfectly clean the dampness will all disappear at the same time. But if there is a particle of dust it will gather in a little zone about that and evaporate there a little later than it does on the clean surface.

The objective is cleaned in the same manner, but since its three lenses are joined in an air-tight mounting it is scarcely possible for dust to collect between them. So we shall have to clean only the upper and under surface of the lens-system. Usually it may be done as with the oculars. But since the upper object glass is difficult to come at we should prepare a wooden stick with some soft linen bound over the end of it, and with this reach down and clean off the glass. Should the

lower lens get besmeared by the careless use of the reagents it should be at once cleaned by repeated washings with distilled water. Water will suffice in most cases. But if it be necessary to use alcohol it should be done with the greatest caution and celerity. For by employing alcohol we are in the greatest danger of ruining the whole system, by the alcohol penetrating within the mounting of the glasses and partly dissolving the Canada balsam by which the crown and flint glass lenses are cemented together. The cleanliness of the objective may be tested in the same way as the ocular, as indicated above, or by examining the reflected image of a window in the glass by means of a magnifying lens.

For the preservation of the microscope, besides cleanliness, the handling of the screws is of importance. This particularly concerns the matrix of the tube and the screw of the objective-system which fits into it. Since by almost every change in the magnification this must be screwed on and off, and since by putting the objective on a little obliquely, the whole screw arrangement may easily be injured, the following method of screwing on the parts is commended as one certain to work no injury to the apparatus. The objective should be set upon the tube with its thread close up against the end; then it should be turned backwards, as in the act of unscrewing, till one hears the short click which shows that the thread has fallen into its proper place, when the system may be reversed and screwed up.

Further, the greatest care should be used in focussing. With high magnifications the objective is brought very close to the object and must be carefully guarded in the act of focussing, or both objective and object will be ruined. Many microscopists do the focussing in this way. The tube is pushed down by hand, or by the rack and pinion, till it comes very near to the objects and then exactly focussed with the fine-adjustment screw. With the use of low powers this is satisfactory, but with higher magnifications, and in the use of the lower by beginners, the following, reverse, method is recommended. By looking across the stage from the side one can bring the objective down very close upon the preparation, still without touching it, bring it

within the focal distance. Then by hand or by the rack and pinion run the tube upward till the coarse adjustment is reached and finish with the fine-adjustment screw. This method secures perfect safety for both lens and specimen.

Respecting the preparation to be examined, it is clear that both slide and cover-glass should be perfectly clean before it is put under the lens. Permanent preparations should be wiped each time before using, with a piece of linen, and if they are used with an immersion fluid it should be carefully removed after each using.

If a preparation is to be studied for a considerable time without removal from the stage, a glass bell should be placed over the microscope and preparation, which, in case it rests upon the same leather or cloth-covered wooden plate, with the microscope, will effectually exclude the dust.

If one brings the microscope in winter from a cold to a warm room and undertakes to use it at once, the ocular glass becomes dimmed by the condensation upon it of vapor from the body or the atmosphere. It is better, therefore, to bring in the microscope some little time before the work is to begin, and set it near the stove to warm up.

If the microscope is to be for a long time out of use, it should be inclosed in its mahogany case, and put away in some closely shutting cupboard in which is placed a little dish of chlorate of lime. This insures the safety of the steel parts from rust, and prevents the formation of verdigris. Never should the microscope, and under no circumstances should the objectives, be stored in a closet in which the reagents are kept, for out of the closest-stoppered reagent bottle, there will come some vapor of acid, which will at length cause the greatest injury to every kind of optical apparatus.

CHAPTER II.

MICROSCOPICAL ACCESSORIES.

Under this term we include a series of implements which finds frequent use in microscopical investigations. The greater part of them are connected with the optical apparatus of the microscope itself. The most important microscopical accessories of which we shall here speak are the preparing microscope, the apparatus for drawing or photographing microscopic pictures, the micrometer, the polarizing apparatus, the goniometer and the micro-spectroscope.

I. THE PREPARING MICROSCOPE.

(Dissecting or Mounting Microscope.)

The preparing microscope is of use, in order to prepare those objects which have previously been made ready for examination by means of the section-cutting instrument, and need to be examined first with a low power, in order to lay them rightly on the object-slide, or, by means of small needles and knives, to further treat and manipulate.

1. *The Simplex.* The preparing microscope consists essentially of a mounted magnifying glass in the focus of which the slide bearing the object may be brought; beneath this is placed the mirror to furnish the necessary light for the object.

A simple magnifying glass gives comparatively but small magnification. To get strong magnifying power it would be necessary to make the lens with a high superficial curvature, and this would involve a short focal distance and too great proximity to the object to allow a convenient use of the dissecting needles. For this reason we have for a long time ceased to use

simple magnifying glasses and instead use a combination of two or three. By this means we secure a shortening of the focus and greater magnification without diminishing the working distance between the object and the under lens. Combinations of two or three of these glasses are called respectively doublets and triplets.

[For the lowest powers of the preparing microscope, single lenses, either double-convex or plano-convex, of from two inch to one-half inch (51 to 13 mm.) focus are commonly employed. By some of the makers they are so constructed that two may be used together.]

[2. *The Coddington Lens.* For powers as high as one-fourth inch (6 mm.) and as low even as one inch (25 mm.) the Coddington lens, Fig. 34, is available. This is a solid glass cylinder whose ends are ground to spherical surfaces both of which are portions of the same sphere, the center of the curvature of both being identical and situated in the center of the glass. A groove is ground around the circumference of the cylinder and blackened to serve the purposes of a diaphragm. This construction gives an excellent definition which is less dependent than in any other magnifier on the exact adjustment of the lens; since, having an optical axis in any available direction, oblique rays pass through it under exactly the same conditions as axial ones, and the performance is therefore not marred by imperfect centering. The working focus is rather short. To partially remedy this fault and to render the instrument less clumsy, the cylinder is often shortened, at a slight optical disadvantage, by bringing the convex surfaces nearer together than when in their theoretical position.]

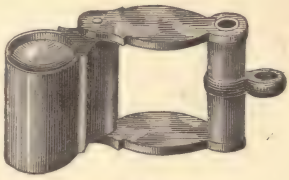


FIG. 34.

[3. *The Achromatic Triplet.* When a magnifier having the external form of a shortened Coddington is made achromatic, it consists of two double convex or meniscus lenses of large curvature, whose aberrations are corrected by a thick lens of glass of a different refractive index cemented between them with Canada balsam. The "Globe lens" of Gundlach is literally an achromatic Coddington, as it is a sphere of flint glass,

ground hollow and filled and corrected by a much smaller sphere of crown glass, the whole being reduced to a cylindrical form by cutting away the unused peripheral portion. Being achromatic it does not require the diaphragm groove. A more common combination is a pair of double convex crown glasses corrected by a thick, double concave flint glass; though some makers, notwithstanding the disadvantage of exposing the softer glass to outside wear, prefer to place the flint, in the form of a meniscus, at each end of the crown. This combination, more or less modified and variously named by different makers, constitutes the achromatic triplet which is now taking the place of all other magnifiers, especially for the higher powers, in the simple microscope. It gives a long working focus and a broad, clearly defined and beautifully lighted field of view, which is a luxury for all purposes and may be considered indispensable for very fine or difficult work. It is usually mounted like the Coddington, Fig. 34. The achromatic objective of the compound microscope is not equally suitable for use in the simple microscope, though the low powers, if mounted short, are sometimes so employed. For this purpose the separating objectives in short tubes, Fig. 2, are available.]

[4. *The Engraver's Glass*, consisting of a pair of plano-convex lenses, about 45 mm. in diameter, mounted in a deep, hard rubber cell, as represented in Fig. 35 and forming a doublet of great size, gives a large field of view, is used with little strain or fatigue to the eye, and is a very serviceable preparing microscope when only the lowest powers are required. The writer has employed these glasses for twenty years with great satisfaction in the examination of pressed plants mounted upon paper as herbarium specimens, in the preliminary examination of hand-writing, and

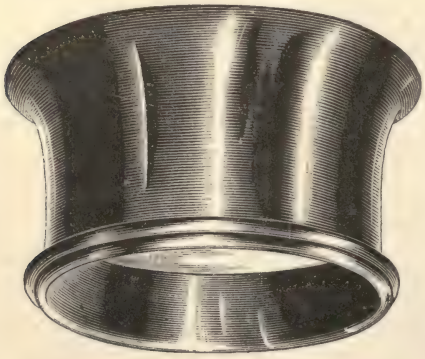


FIG. 35.

in the selection from among large masses of material, as of fabrics or mixed fibers or other substances supposed to contain inequalities or adulterations, the portions requiring further investigation; also in performing under the lenses such manipulations or dissections as require only a low amplification. Such lenses may be best supported upon the large lens holder shown in Fig. 37.]

[5. *The Handy Dissecting Microscope.* A preparing microscope of extreme simplicity, made by Bausch and Lomb, is shown at $\frac{2}{3}$ its natural size in Fig. 36. It consists of a glass plate into which is screwed an upright brass rod *B*, which supports the magnifiers at *A*. These are three simple magnifying glasses, capable of being used singly or together, and mounted

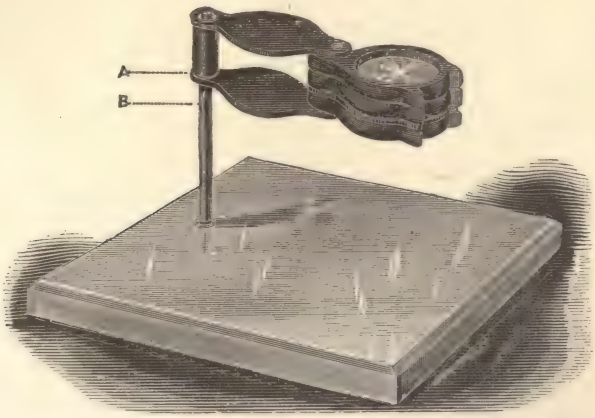


FIG. 36.

in a form available for ordinary pocket use. The addition of a Coddington lens, Fig. 34, suitably mounted to be attached to the same stem, gives a somewhat higher power, of good quality.

[6. *The Lens Holder.* Finding the lens holders in use to be of too limited applicability, being too light, for instance, to carry the large engravers' lenses, and too short-armed for the convenient study of handwriting upon large sheets of paper or of mounted herbarium specimens, or else too unstable for use with higher powers, the writer has devised and employed a

form, arranged somewhat like the stands used by engravers, which is (unlike them) sufficiently firm and manageable for either large or small magnifiers, of low or high powers, and is available for an arm-length of 20–25 cm. It consists, as shown in Fig. 37, of a rectangular frame which slips over the pillar of a bull's eye stand, both it and the bull's eye being often mounted upon the same stand, for the sake of simplifying the apparatus, and because they are often advantageously used in combination. The frame slides smoothly up and down the pillar, being held in any position by an included spring. To an

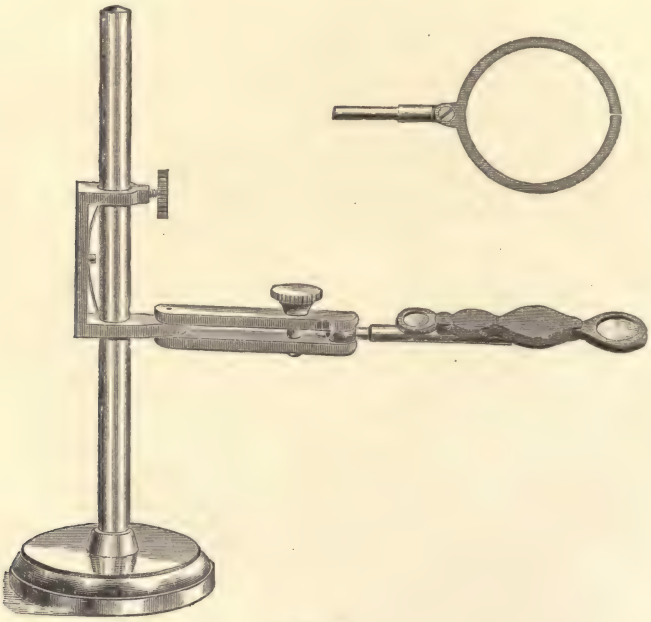


FIG. 37.

extension of the bottom of the frame is attached a horizontal arm, having first a horizontal pivot joint, and secondly a ball and socket joint, the tension of these being readily adjustable by means of a screw with a large milled head. By bending the joints, the lens may be brought near the pillar for use in connection with the bull's eye; or by attaching the jaws or ring to a longer wire, the total arm length may be increased at will.]

[At the end of the arm rises a vertical pivot upon which can be slipped almost any kind of pocket magnifier, such as a Coddington, or achromatic triplet Fig. 34, or a three-lens system like *A*, in Fig. 36, or a double bellows-shaped arrangement like that shown *in situ* on the arm. Or, the lenses being removed, a split wire may be inserted into the hollow end of the arm, bearing a pair of hinged semicircular jaws, shown in the figure, for carrying an engraver's glass, or any variety of large lenses not requiring delicate adjustment. For magnifiers of higher power, requiring more precise adjustment, a ring is substituted for the jaws.]

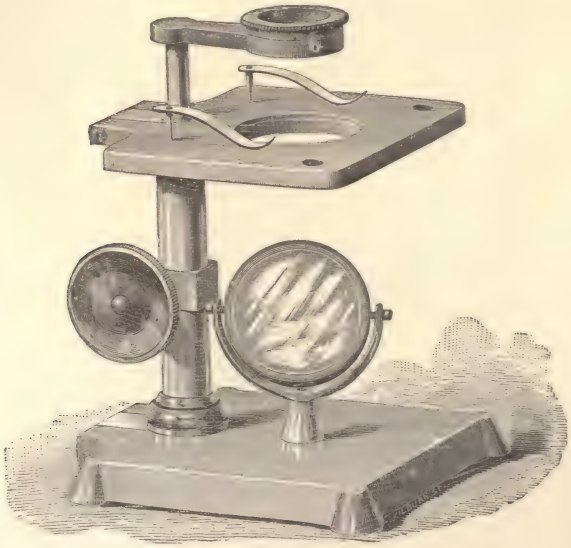


FIG. 38.

[There is a fine adjustment at the top of the rectangular frame, where a screw with milled head, pressing the pillar against the spring, promptly but steadily depresses the lenses to the extent of about four times its own motion.]

[This apparatus is now made for the trade by the Bausch and Lomb Optical Co. When supplied with fine achromatic lenses, and kept standing always ready upon the table, it becomes con-

stantly useful even to persons well supplied with elaborate apparatus. It is worked, if transmitted light be required, over the stage of any dissecting microscope that may be within reach. By turning the jaws or ring into a vertical position, it is well adapted to the examination of living aquatic plants in a glass jar or aquarium; for which purpose powers of 50 to 100 diameters may become available by using the Brücke magnifier

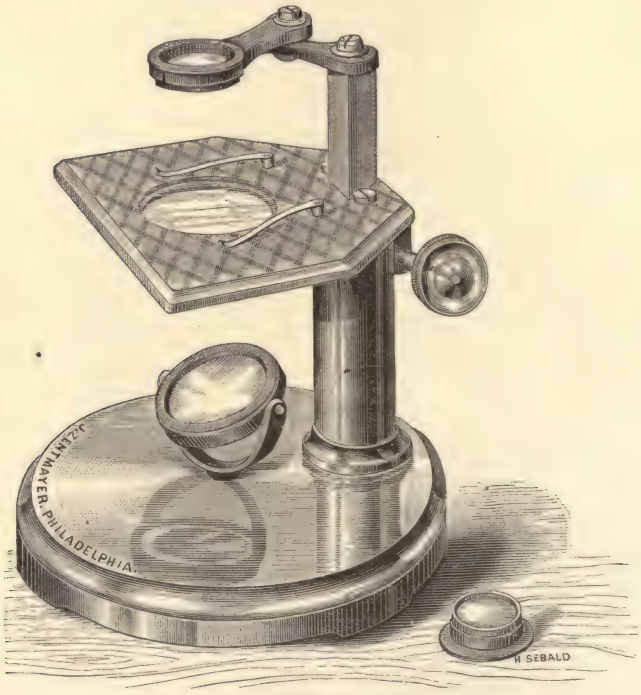
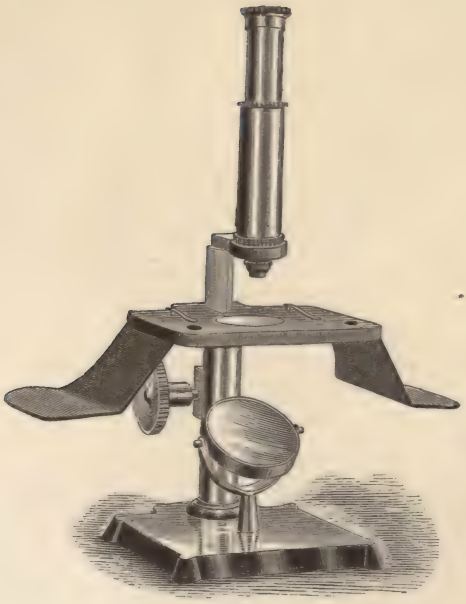


FIG. 39.

(p. 107) or the Bausch and Lomb compound dissecting magnifier (p. 108), which for this use should be screwed, not slipped, into the ring.]

[7. *The Compact Dissecting and Mounting Microscope* is made by Bausch and Lomb, and is a preparing microscope of medium size and cost, and of sufficiently portable form for pocket use. It is represented in Fig. 38. A japanned iron base, 9 cm. square, carries a mirror in a central location for axial



Compact Compound Dissecting Microscope. Bausch & Lomb.

illumination, and also supports a pillar which carries, at a height of 9 cm., a stage of blackened brass, slightly smaller than the base. This stage has a central opening of about 32 mm., supplied with a removable glass plate. Inside the pillar is a triangular rack-bar moved by a pinion with a milled head, which carries a transverse bar with a ring at the farther end, into which, in the optical axis of the instrument, the amplifying lenses are inserted. This ring is also furnished with the society screw, by means of which objectives may be substituted for the simple lenses. The mirror can be instantly transferred to the bottom of the stage for oblique, or to the top of the stage for opaque illumination. Hand rests after the German style can be attached to the sides of the stage, as figured in Plate XII. Both base and stage can be folded flat against the pillar, in order to be packed in a very small case.]

[8. *The Botanical Dissecting Microscope*, Fig. 39, made by Mr. Zentmayer, is a somewhat larger and heavier instrument than the one just described; but it has essentially the same parts similarly arranged. The circular base is 12 cm. in diameter, and the stage is 9 × 12 cm., with a central opening 4 cm. With the stage increased to 11 × 15 cm., and a larger mirror substituted, compactness would be still further sacrificed, but the working capacity would be, in the writer's opinion, correspondingly increased.]

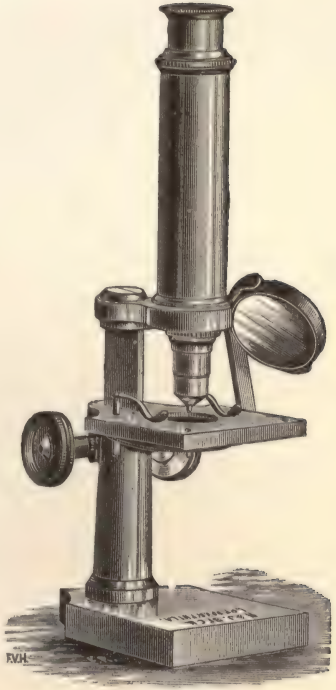
[9. *The Brücke Compound Dissecting Microscope.* A separating achromatic objective, whose lenses can be used either singly or together, with an ocular in the form of a concave eye lens (as originally proposed by Prof. Brücke of Vienna) inserted into a small tube sometimes less than 5 cm. high, which acts as a little compound body, constitutes a microscope, upon the principle of the Galilean telescope, which has long been used in France and Germany, and to a more limited extent but not with less satisfaction, in this country. The objective lenses commonly used, by themselves give powers of from 12 to 30 diameters, which powers are increased by the eye lens to from 40 to 100 and upwards. With the highest powers the working focus is exceedingly long (8 mm.), affording ample room for the use of needles or dissecting instruments. The field of view is rather small.]

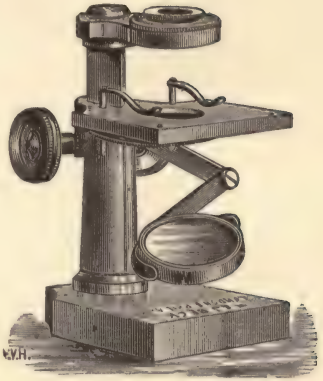
[10. *The Bausch and Lomb Compound Dissecting Microscope.* This form, just introduced as a substitute for the Brücke instrument, consists of a little compound body (combined with their compact microscope in Plate XII) only 75 cm. long and 19 mm. in diameter, which contains a diminutive objective, ocular and erector. By the use of the draw-tube the erector can be carried from near the objective to near the top of the body, giving a range of powers of from 12 to 150 diameters, the working focus meanwhile varying inversely from 38 to 6 mm. The light transmitted is less than with the Brücke apparatus, the field of view averaging about the same for the same powers. The advantage of the new arrangement is the ready command of the whole range of intermediate powers by simply sliding the draw-tube. For the higher powers, it may be used without the erector if preferred. The objective is a dividing one, whose lenses, the compound body being removed, can be used separately or in combination as simple magnifiers.]

[11. *The Histological Dissecting Microscope*, a combined simple and compound microscope made by R. and J. Beck of London* and Philadelphia, figured in Plate XIII, is adapted to a considerable range of family, school and amateur use. The simple or preparing microscope figured at the right, is small, compact and substantial; and is somewhat suggestive in form of the Zeiss preparing microscope. It is promptly converted to a compound microscope as shown at the left, for examinations requiring higher powers, by removing the lens from the transverse arm, and by replacing it with a small compound body. As a simple microscope this instrument lacks breadth of stage, and as a compound, it lacks a fine adjustment; still it is used with much satisfaction by many whose wants do not demand a higher grade of apparatus.]

[12. *Hand Rests.* In using any form of preparing microscope, not even excepting those with the largest stages, much increase both of comfort and of steadiness in manipulation can be secured by using hand rests at the sides of the stage. Thin

*This instrument, as well as some others mentioned hereafter, though English, is admitted among American apparatus, for the reason that the American business relations of the London house have been such for several years past, that the Beck firm has come to be regarded as partly an American enterprise, and that their wares have become as familiar and accessible as if actually made in this country. R. H. W.





Beck's Combined Histological and Dissecting Microscope.

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metal wings, as in Pl. XII, can be attached to the stage for this purpose. For frequent and prolonged use, however, a broader and firmer support made of wood and resting upon the table instead of the stage, is more restful and is coming into use. The writer has been accustomed to use a rest, made of mahogany strips about 1cm. thick, and 10 to 12 wide, constructed as shown in front view, somewhat diagraphically, at about one-fourth size in Fig. 40; where there is a base lying

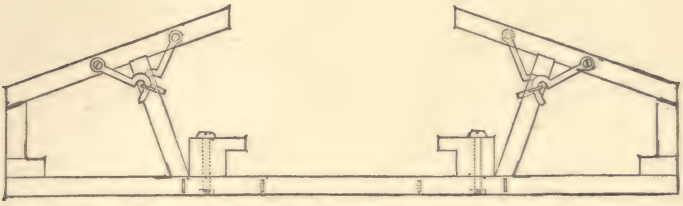


FIG. 40.

upon the table, the rests attached at one end by hinges and held down firmly with brass hooks, hinged strips supporting the rests at the desired height and in an inclined position, and wooden buttons held by large screws (which for better stability should be fastened with brass nuts below) for holding the base of the microscope firmly in position. The hinges are all so arranged that the strips can be folded together solidly, for portability, as shown in Fig. 41, and held snugly in

FIG. 41.

that position by the same hooks as when open. The hooks are on the farther side of the wooden strips. Such an arrangement can be purchased from the microscope dealers, or made for one's own use by any person fond of such experiments. By a slight change in size it is applicable to any preparing microscope. It should be made of such size that the upper ends of the rests

will be nearly continuous with, or slightly below, the stage of the microscope. Exact approximation is not necessary. When properly adjusted the rest is perfectly firm and steady. When portability is not required, the hinges and hooks may be dispensed with, and the wooden strips fastened together with glue and brads. R. H. W.]

II. APPARATUS FOR DRAWING MICROSCOPIC PICTURES.

If we suppose that *m*, Fig. 42, be the tube of a microscope, *d* the objective and *c* the ocular, then the preparation *o* lying on

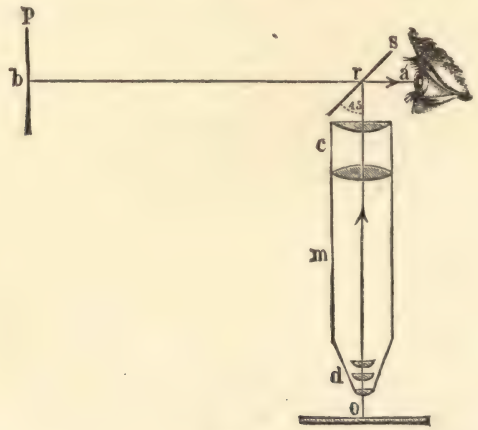


FIG. 42.

the stage sends out a bundle of rays which, as the mathematical line *or*, passes out of the ocular in the vertical direction, *or*, in the way already described. Now place a small glass mirror *s* directly over the ocular, inclined at an angle of 45° to the emitted rays, and the rays will be reflected at a like angle from the mirror, changed now from a vertical to a horizontal direction. If I put my eye at the point *a*, I shall naturally perceive the microscopic image reflected in the mirror. If now the mirror be transparent and a sheet of paper *p* be fixed up perpendicularly

behind it, I shall see through the mirror and look upon the paper beyond and the microscopic image will appear to be lying on the paper, or, in other words, it will be projected through the mirror upon the paper. If we choose, the distance [a b = 10 inches (25.4 cm.), the magnifying power, which is really a question of angular extent, will be always converted to linear measure at a fixed distance, as it should and must be to render a variety of records by different observers comparable with each other. The magnifying power represented by comparing the size of the drawing made at this standard distance with the actual size of the object itself will also represent more accurately than at any other distance the resolving power of the instrument; since the power of the microscope to render small objects or fine points of structure distinguishable depends on the angular size of the object as seen in the microscope compared with that of the object, not as unseen by the naked eye upon the microscope stage, but as it could be seen by the naked eye at the best distance that could be chosen for that purpose. By common usage this distance is established at the standard limit of 10 inches (25.4 cm.) which is assumed to be an average representation of the distance, varying for different eyes, of most distinct vision for small objects. The impropriety of the advice, which does not lack high authority, to project or draw the magnified images, for measurement or comparison, at the exact distance of the object on the stage becomes evident in such extreme cases as using a simple microscope, with the object very near the eye, or a compound microscope having a tube two or three inches or as many feet in length. R. H. W.]

One may observe the working of this simple contrivance, very easily in the following way. Fix a clean cover-glass of the utmost possible thinness to the top of an ocular, by means of a drop of wax, in such a position that it will meet the rays of light from the objective at an angle of 45°, Fig. 42, *s*. Powerfully illuminate the field of view, and put on a low magnifying power; then the image of the object beneath will be seen projected, and somewhat darkened, on the paper *b*. With a pointed lead pencil, one may then easily trace the coarser outlines on the paper, since one can see at the same time both

the image and the pencil point. This simple contrivance would be perfectly satisfactory for tracing microscopic images, were it not for two faults. First, the paper surface is in a very unfavorable position, the hand having no support, hence the tracing will be in coarse, rough outlines, and secondly, the image is very poorly lighted. Also, according to the investigations of Fresnel, in the reflection of a transparent mirror, the 0.944 part of all the rays that fall upon the glass pass through it and only the 0.056 part are reflected and come to be of value in the reflection-image, so that this will have but the $\frac{1}{18}$ part of the brightness of the original image. These prevailing faults can be overcome in great part in the following way.

In order to project the image on a horizontal surface it is necessary [to place the microscope body horizontally while drawing, or else] to have a contrivance for double reflection. This is represented in Fig. 43, where *m* again is the microscope-tube bent at a right angle at *r*, where there is a small thin-glass mirror *s* placed at an angle of 45° to the ray *or*. The ray on striking this is reflected in the direction of *rr'* through the ocular *c*. Here again a transparent mirror is set at an angle of 45° which reflects the rays in the direction *r' a*, and the image will appear to the eye to be projected on the horizontal surface beneath at *b*, where, on the already prepared paper, the tracing may be conveniently done.

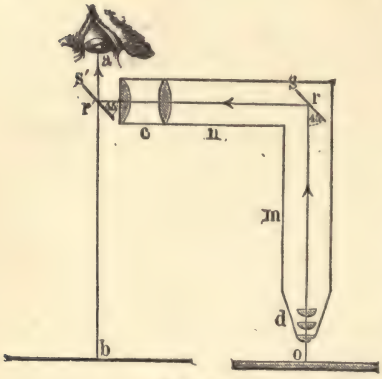


FIG. 43.

One can easily construct this contrivance himself, by using a paper tube bent at *r*, at a right angle and made to fit on over the microscope-tube, and into which an ocular can be put. The mirror *s*, which should be made of the thinnest glass, may be blackened with India ink on the back side. By making the magnification low, and the light strong, this simple apparatus is very well adapted to the purposes of microscopical drawing. In

order to save the great quantity of light which passes through the transparent mirror, s', not reflected, a small metallic mirror is substituted, which not being transparent reflects all the light that falls upon it. It should be made of silver, and supported on a very slender arm. It must also be smaller than the pupil of the eye, in order that the eye may look by it and perceive at the same time the underlying paper, pencil, etc., and the image have the appearance of being projected upon the paper. Sömmering[1] invented the small metallic mirror as an aid to microscopical drawing.

The use of the reflecting glass mirror has always had this disadvantage that it did not give a perfectly sharp image, and for this reason. We will suppose A B C D, Fig. 44, to represent a magnified section of a glass mirror blackened upon the back. On this mirror the light ray $o\,n$ falls at an angle of 45°. Here a part of the ray undergoes reflection, in consequence of which this part of the beam of light takes the direction $n\,m$ (angle $A\,n\,o = B\,n\,m$). Another part of the beam, however, passes through the glass being refracted by it in the direction $n\,n'$ to the back side of the glass. Here it is reflected so that angle $C\,n'n = D\,n'n''$. Here it again suffers refraction at the front surface A B, and in consequence goes as a ray in the direction $n'\,m'$ parallel with the ray reflected from the front surface $n\,m$. By the use of the glass mirror, therefore, the ray will be divided into two rays which run near and parallel to each other, and whose distance apart will be greater in proportion to the thickness of the glass used. So the image reflected from a glass mirror will be divided into two images which are not exactly superimposed, and the consequence is, the image is less distinct after reflection than before.

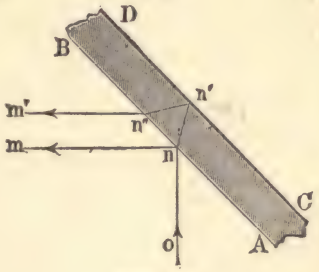


FIG. 44.

This evil is obviated by the use of a reflecting glass prism, represented in section as a right-angled isosceles triangle,

[1] See H. v. Mohl, Mikrographie, p. 324.—Harting, Das Mikroskop, pp. 176, 901.

which makes the reflection from the hypothenuse. The latter must be ground absolutely flat, which is by no means an easy thing to do. Fig. 45 represents two reflecting glass prisms which are so arranged as to correspond to the two glass mirrors in Fig. 43, r and r' in both figures corresponding to each other. We will suppose that the ray o passing in the direction of the arrowpoint falls upon the side of the prism p perpendicular to its surface. It enters the glass unrefracted and passes to r, striking the hypothenuse surface at an angle of 45°. Here it suffers a total reflection and takes the direction rr'. In like manner it enters the second prism p' and is likewise reflected at r' in the direction $r'a$. It is therefore clear that the two mirrors s and s', Fig. 43, can be replaced by the prisms pp'.

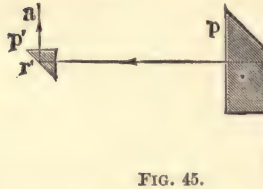


FIG. 45.

FIG. 46.

[A prism like p', but about half the size figured, is sometimes cemented to the end of a small bar and mounted in front of the ocular, the large prism being dispensed with. It is very convenient for drawing either in the horizontal or inclined position of the tube; but as it inverts the image, as do other singly-

reflecting arrangements, difficulty is experienced in retouching the sketch made by its use. It also requires great steadiness of position on the part of the user. By substituting for the little prism a small steel mirror, also smaller than the pupil of the eye, the Sömmering mirror is produced, which acts precisely like the small prism and has much the same advantages and defects. Neither of the above has come into very general use.]

[1. *The Neutral Tint Reflector.* The singly-reflecting camera, called the neutral tint reflector, was brought into use at the suggestion of Dr. Beale of London and is shown *in situ* in Fig. 46. This reflector was originally a thin plate of neutral tint glass, but a common white cover-glass is now employed as a satisfactory substitute. It is supported in front of the ocular at an angle of 45°, so that the observer looks obliquely through it at the paper and pencil, seeing at the same time and apparently in the same place the microscopic image reflected from the glass. The glass being thin, but little indistinctness results from the confusion of the separate reflections from its two surfaces; while the inconvenience of its inverting the image seems to have been quite overbalanced by its simplicity and cheapness, and the facility with which it can be used even by inexpert persons. Though practically limited, for the reason stated above, to those uses which allow a nearly horizontal position of the microscope body, this contrivance is probably more used in this country than any other form of camera lucida. R. H. W.]

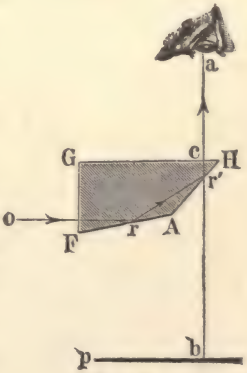


FIG. 47.

2. *The Wollaston Camera lucida.* The little mirror *s* in Fig. 42, *s'* in Fig. 43, and the prism *p'* in Fig. 45 may be replaced by an apparatus invented by Wollaston and represented in Fig. 47.[2] It consists of a four sided glass

[2] See Wollaston in Phil. Transactions, 1809, No. 38, p. 741. — W. H. Wollaston's description of the camera lucida, an instrument designed for sketching objects in the neighborhood, and for making magnified or minified tracings (Gilbert's Annalen der Physik. Bd. XXXIV. N. F. Bd. IV, 1810, pp. 353-361, I Tafel) — Gehler's Physikalisches Worterbuch, Leipzig, 1825, Bd. II, p. 30 *ff.*

prism *A F G H*, in which *FGH* is a right angle while *HAF* equals 135°. The ray strikes the surface *GF* in the vicinity of *F* perpendicularly, so that it passes unreflected through the prism to *r* and then to *r'* in each undergoing total reflection, and passing out of the prism in the direction *r'a*. The eye placed at the point *a* looks downward through and just beyond the edge of the prism at *H*, and perceives the sheet of paper lying at *p*, on which at *b* the image of the rays seems to be.

[The Wollaston camera lucida is usually attached to the microscope by means of a spring-ring slipping over the top of the ocular. Connected with the front end of this ring is a light brass box containing the prism and wholly covering it except that the side *FG* in Fig. 47, is left unprotected and that the edge *H* is exposed (more clearly seen at *F*, Fig. 48), by a notch through which the observer looks down upon and through the prism, at the same time that he views the paper and pencil with that portion of the pupil of the eye that is not over the prism. This camera lucida, originally devised for general drawing, is still used more than almost any other for microscopical work. Care is required as in the use of other cameras, to so regulate the intensity of the illumination of the field of view and of the drawing paper that neither shall be obscured by the relative brightness of the other.* For greater ease and distinctness in viewing the paper, a lens of long focus, like a spectacle glass, is often placed below the prism, just below *r'*, Fig. 47, and in the line of the ray *ab*. This camera is generally used with the body of the microscope in a horizontal position, and with the paper lying horizontally beneath the ocular, since a vertical

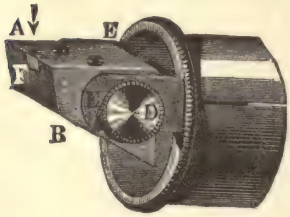


FIG. 48.

* As the light in the field of view and on the object may be easily and exactly regulated by means of the diaphragm and mirror, the principal difficulty will be found in the management of the light upon the paper. Hence a small movable screen of thick paper, which one may easily contrive for himself, about 30 cm. long and 20 cm. high, placed more or less directly between the source of light and the paper, thus darkening the latter at will with its shadow, will be found very useful in conducting this kind of microscopical drawing. A. B. H.

position of the tube would require the paper also to be vertical. R. H. W.]

3. *Nobert's Camera lucida.* Another camera lucida originated with Nobert and is diagrammatically represented in Fig. 49. It permits the picture to be drawn on a horizontal surface. The rhombic prism, *ABCD*, bears on its oblique surface *AB* a small right angle prism *EFG*, made fast to the surface *AB* at *EF* by means of Canada balsam. It is then so placed over the ocular of the microscope that a ray of light proceeding from it strikes the surface *FG* perpendicularly. It goes directly through the mass of glass to *a* in the direction *oa*, unrefracted and losing little light by reflection at *r'*. Again ray *b* from a horizontal drawing surface strikes the oblique side *BC* of the rhombic prism and passes through to the side *CD* at *r* and suffers a total reflection in the direction *rr'*. At *r'* it is again totally reflected in the direction *ra*, and reaches the observer's eye at the same time and in the same direction with the image-forming rays from the microscope *oa*. The observer sees in the microscope, by means of this apparatus, not only the object but at the same time also an image of the drawing surface and pencil.

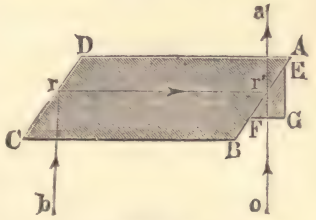


FIG. 49.

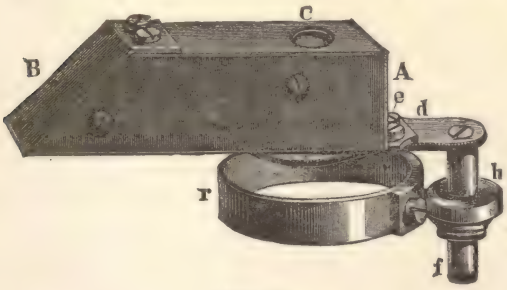


FIG. 50.

The Nobert drawing prism as completed by Nachet [and known in this country as Nachet's] is represented natural size in Fig. 50. This apparatus for horizontal drawing consists of a metal ring *r* which is put upon the microscope-tube and the ocular is replaced. The metal ring bears a projection *h*, into which the brass rod *f* exactly fits, and not only turns upon its axis but likewise may be moved up and down by hand. On *f*

is fastened a blackened metal plate *d*, which widens into a circle over the ring and has its center bored out with a large opening. The metal box *AB* is fastened to this plate by means of the screws *e*. The box contains, within, the two glass prisms more exactly illustrated in Fig. 49. At *C* and in the corresponding place of the under surface is a circular opening which falls in with that of the plate *d* just now mentioned. The box must be so large that *B* shall extend laterally beyond the foot of the microscope. Directly under *B* is the surface of the paper on which the drawing is to be done. The eye looks down through *C* and sees in the field of the microscope the drawing paper and the pencil. The box *AB* may be turned aside at will and leave the ocular free.

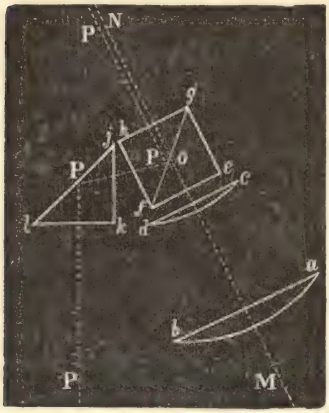


FIG. 51.

[4. *Grunow's Camera lucida.* Mr. J. Grunow has contrived a camera lucida in which an effect nearly identical with that of Nobert and Nachet is secured, but in a slightly different manner. This device is shown in section in Fig. 51 where *P* is a rectangular reflecting prism like those in Fig. 45, while *P'* is a cube formed of two such prisms cemented together with Canada balsam. One of the surfaces of contact, *fg*, is silvered, except a circular spot in the center about half the diameter of the pupil of the eye, thus bisecting the cube obliquely with a perforated mirror. It is evident that the eye at *PN* can look directly down the microscope-tube in the direction *NM* through the cube *P'* by reason of the central aperture in the silvered surface *fg*, while the paper on the table at *P* can be seen with ease at the same time by reflection from the glass surface *P* and from the silvered portion of the interior surface *fg*. This device is mounted and slipped over the ocular. It is used with the same comfort to the eyes as the Nobert form, though not with the same position

of the microscope, since the Grunow camera is best adapted to an inclined position of the microscope-tube, making the line *PP* vertical and the drawing upon the table free from distortion when the tube is inclined at about an angle of 30°, while Nobert's gives the same results in a vertical position of the microscope, and requires an inclined drawing board when the tube is inclined.]

[5. *Photo-micrography.* The substitution of the *camera obscura* for the camera lucida as a means of preserving or reproducing microscopic views, while not without disadvantage in respect of showing the relation of parts, and of selecting typical points in different portions of the field and combining them all in one picture, is often desirable on account of the impartiality and completeness of detail secured by the automatic action of the light itself. Hitherto its use has been mostly confined to the few who happened to possess an exceptional access to, and familiarity with, the mysteries behind the scenes of some photograph gallery, and to the still smaller number who were prepared to incur the expense of employing the assistance of a professional photographer. Recently, however, the development of amateur photography as a popular pastime has placed within reach of the microscopist the means of doing this work for himself, without previous experience, unusual mechanical skill, or considerable expense.]

[The Bausch and Lomb Optical Co. offer for sale a very compact and beautiful amateur photographic camera, suitable for general work, with a simple attachment by means of which it can be brought into relation with the possessor's microscope, and the magnified image of an object focussed upon its sensitized plate. Mr. W. H. Walmsley of Philadelphia, late American Manager for the Messrs. Beck, has arranged, and has introduced to the trade, a complete outfit, containing not only a camera, and platform for connecting with the microscope, but also the requisite chemicals, and a complete, carefully selected assortment of the various supplies required for the work. This will be a convenience to those who do not wish to incur the trouble and delay of learning their own wants by means of their

own experience. Mr. Walmsley's arrangement of his apparatus is shown in Fig. 52, which illustrates clearly the theory and practice of such devices generally. R. H. W.]

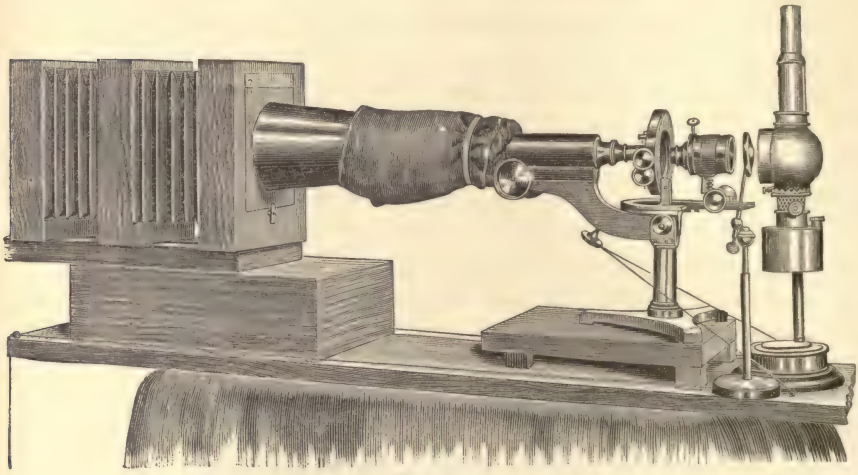


FIG. 52.

III. THE MICROMETER AND MICROSCOPICAL MEASURING.

In microscopical investigations it is often a matter of great importance to be able to determine the true size of an object under the microscope. This can be done in various ways. But as different as the various apparatus is which has been designed for this purpose, there are but two principles involved in it, viz., either to measure the object itself, or, to measure the magnified image of it which the objective produces. The apparatus which aims to do the former, we call "objective micrometers;" that which aims to accomplish the latter we name "ocular micrometers." Objective as well as ocular micrometers may be either glass- or screw-micrometers, that is their essential part may be fine diamond-rulings on glass, or a carefully cut screw whose thread has a definite height.

I. Objective Micrometers.

A. The Objective Glass-Micrometer. (Stage micrometer.) This consists of a small glass plate on which, by means of a diamond, fine rulings are cut, a millimeter being divided into one hundred equal parts. [Its appearance under the microscope is as represented in Fig. 53. Subdivisions of the inch, or inch and millimeter compared, are also used.] If such a ruled plate be put under the microscope in place of the object slide and the object to be measured be laid upon it, the length of the latter can be determined. This kind of microscopical measuring is doubtless the simplest in the world, but, alas! there come in so many disturbing influences that its use is limited to a few cases only. [It is chiefly used to determine the working value of the divisions of the ocular micrometer.]

B. The Objective Screw-Micrometer. [This apparatus is a sliding plate attached to the stage, or constituting a part of the stage itself, by which the object while in view in the microscope is carried steadily past a fine line fixed in the ocular. The movement is accomplished by the push of a fine horizontal screw acting upon the plate, and the distance traversed in carrying the whole object across the line, the same being the diameter of the object itself, is determined by the number of the turns and fractions of a turn of the screw, the width of whose thread is very accurately known. Though manufactured with great skill, and theoretically capable of reading off results with almost unlimited minuteness, directly and without the trouble of computations pertaining to ocular micrometers, this apparatus requires great skill in manipulation and is particularly liable to get out of order. At best it is believed to be inferior to the ocular micrometer in accuracy, since it measures the object directly, and not its magnified image; any errors in its performance being, therefore, multiplied by the whole magnifying power of the microscope, instead of by the power of the eye lens only. For these reasons it is, as conceded by the author, giving place more and more to the ocular micrometer.]

[Some microscopes with a mechanical stage have the adjacent surfaces graduated, with or without a vernier, so that the position of the stage or the distance it has moved can be read off and recorded. This serves as a finder, by which the position of an object mounted on a specified slide may be registered, and supplies a means of roughly measuring the size of large objects, as for instance, the width of a leaf or the length of an anther. R. H. W.]

2. The Ocular Micrometer.

Ocular micrometers by which we undertake to measure the real image of the object are either glass- or screw-micrometers. They have, however, this advantage over the objective micrometer, that they do not require by far, the same precision of work as that, since a casual error in the rulings, etc., has a magnitude in the results of the measurement, equal only to the given error divided by the whole number of the objective magnification.

A. Ocular Glass-Micrometer. This apparatus has a wider distribution in Germany than all other micrometers. It consists, as the objective glass-micrometer does, of a glass plate upon which are diamond-rulings. A thin cover-glass is put over the rulings for their better protection. The scale extends for four or five mm., and each millimeter is divided into ten or twenty equal parts, the fifth and tenth line being extended in the ordinary way for greater convenience in counting.

[Whether it be divided into metric or English units is unimportant, except that it may sometimes be required for use as a stage micrometer, since the working value of the divisions depends upon the optical conditions under which they are used, and must be carefully determined after the apparatus is arranged and in order for the proposed work.]

[The simplest, cheapest and commonest form of ocular micrometer is a round cover-glass, properly ruled, which is laid when about to be used upon the diaphragm of the ocular in the focus of the eye lens. The rulings should be turned downwards to

bring them close to the diaphragm, and if not distinctly visible, they should be made so by slightly unscrewing the lens, and thus bringing them into its focus. When properly adjusted, the ruled scale will appear clearly defined in the field of view somewhat as represented by Fig. 53. Sometimes the glass is cut away at one end of the lines, leaving them at the edge of a half-circular disk; and thus, without impairing the micrometer, half the field is presented with its definition undisturbed by the additional glass plate. Some makers mount the glass scale in a plate of brass or hard rubber to be slipped into the ocular just above the diaphragm through slits left for that purpose; the slits being closed by a short, sliding tube, when the micrometer is not in use. Unless a screw be attached, as is sometimes done, giving a delicate lateral movement, the use of any ocular micrometer is much facilitated by the employment of the mechanical stage, by means of which a coincidence of certain lines with any part of the object to be measured can be the better secured. R. H. W.]

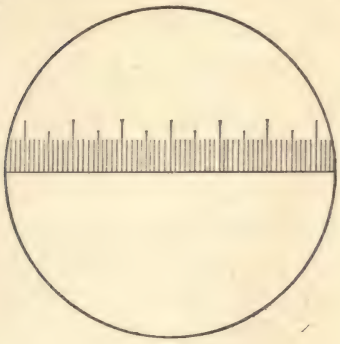


FIG. 53.

Measuring by means of the ocular micrometer is conducted in the following way. The micrometer is put in place, its scale and the object brought in focus, and then by turning the ocular and moving the object, the whole length of the object to be measured is covered with the scale, in such a way that its rulings are perpendicular to the longer axis of the object. Then adjust the object as sharply as possible and count up how many of the rulings are covered by it (whole and fractions). This number is to be noted down and if one desires very great exactness the measurement may be repeated on different parts of the scale and the results averaged.

It is clear enough that one does not get the exact and real size of the object by this process. To get this one must either exactly determine the number of magnifications of the objective-

system together with that of the field glass and the eye lens, so as to ascertain the true value of the ruled spaces of the micrometer, or he must determine their true value by the use of an exact objective glass-micrometer. If he has for instance found that in the first case, the objective and the field lens together magnify seventy diameters and the rulings of the micrometer are 0.05 mm. apart, so by the use of that objective-system the space between two rulings will amount to a magnitude in the object of 0.714 of a micromillimeter. The determination of the relative value of the ocular micrometer by means of the objective glass-micrometer is very simple. The latter is used as an object, and then it is determined how many of its divisions answer to a certain number of the divisions of the ocular micrometer. Divide the first by the last and the result will be the true value of the ocular-micrometer divisions in units of the objective micrometer. If, for instance, 20 divisions of the ocular micrometer cover an extension of 87 micromillimeters of the objective micrometer, the value of an interval in the ocular micrometer will be $\frac{87}{20} = 4.35\mu = 0.00435$ mm. [In such work, round numbers are always preferred, for the sake of simplifying the labor of computation and comparison. In the above example, for instance, the 20 divisions of the ocular micrometer could, by lengthening the optical axis of the microscope by slightly raising the draw-tube, be made to cover 100 micromillimeters instead of 87, the value of one interval becoming 5μ instead of 4.35μ, and the labor of computation being reduced to almost nothing. So in the author's following illustration, the values 8, 3.63, 1.27, 0.55 would by a judicious use of the draw-tube be changed to 8, 4, 1.5 or even 2, 0.6 or even 1 respectively. Having once found a convenient position of the draw-tube for a certain purpose, much trouble will be saved by recording that position so that it can be promptly restored when next wanted. The draw-tube should be graduated for this purpose. Having been set in position, approximately, by means of the graduation, a slight readjustment will instantly secure exact apposition of the desired lines (say 20 to 100) of the ocular and objective scales. R. H. W.]

If the manufacturer would take pains to determine the micro-

metric value for every system furnished it might be designated [approximately, but not with sufficient accuracy for fine work] on the tables of magnification which accompany the microscope. If this were given, as we may suppose for illustration, for the systems I, II, III, IV, of an instrument 8.00, 3.63, 1.27, 0.55, respectively, then I have these numbers by which to multiply the number of units read off from my ocular micrometer in order to determine the true size of the object measured in micromillimeters.

Thus, the object being measured is found to cover 17 divisions of the ocular micrometer, the magnification being with the system I. It has consequently a true length of $17 \times 8. = 136\mu$; or, the magnification is made by system IV, the object covering 53.5 divisions of the micrometer. Its true size will then be $53.5 \times 0.55 = 29.4\mu = 0.0294$ mm.

Some firms have lately furnished adjustable ocular glass-micrometers, which are provided with a fine screw by means of which the rulings may be moved in a horizontal direction, and a further adjustment by which the rulings may be brought very exactly into the focus of the ocular lens. For fine measurements this is decidedly preferable to the one previously described.

[Objective glass-micrometers, here called stage micrometers, if based upon the English units are commonly ruled to $\frac{1}{10}$, $\frac{1}{100}$ and $\frac{1}{1000}$ inch. The metric (decimal) system, however, is coming into somewhat general use here, and it must be admitted that 1 mm. (about $\frac{1}{25}$ in.) $\frac{1}{10}$ and $\frac{1}{100}$ mm. form a more convenient series than any round fractions of the inch. $\frac{1}{100}$ mm. is, moreover, equal to 10μ, the micron or micromillimeter, μ, being the only recogized unit in the world for a minimum unit in measurements. At present, the metric values being not yet familiar to all students, stage micrometers are often ruled showing the metric and English scales in comparison with each other. The stage micrometer should be ruled with the utmost possible precision, as its errors are multiplied by the whole magnifying power of the microscope employed. It has been known for years that the best micrometers in use contained perceptible errors. The standard micrometer of the National Committee, adopted in 1883 by the American Society of Microscopists,

represents 1 cm. subdivided to 1, $\frac{1}{10}$ and $\frac{1}{100}$ mm.; and the value of its spaces, as related to larger standards of length, have been determined with a certainty and precision not known to have been attained before in any micrometric standard. Copies of this standard can now be obtained from the dealers in microscopes; and the loan of officially certified copies, for comparison, can be obtained, under certain restrictions, from the officers of the society. R. H. W.]

B. The Ocular Screw-Micrometer.

This micrometer is seldom used in Germany but frequently in England, where, however, on the whole, very little and very indifferent microscopical research is made, except indeed, to look at diatom frustules, and to take delight in their markings; but for that, one has there the most expensive and showy apparatus.*

The measuring apparatus in question differs very little from the objective screw-micrometer. By means of a micrometer screw whose drumhead is divided into one hundred parts, a movable thread is carried through the field of a Ramsden ocular toward another thread parallel to it. The revolutions are read off from a metal scale in the field of the ocular.

[This apparatus, borrowed from the telescope, and familiarly known as the "Ramsden" or "cobweb" micrometer, requires to be well made, and to be attached to a sufficiently firm stand. It is also rather expensive. It is believed, however, to be capable of performing a series of measurements with a rapidity and precision not easily attained by any other means. Like other ocular micrometers, it is most readily used upon a microscope having a mechanical movement to the stage.]

[Fig. 54 shows a cobweb micrometer as now constructed by

*The preceding remark is retained out of respect to the author's liberty of opinion, and as a very vivid caution (which might be useful also in this country) against a real and conceded evil; but with a very decided belief on the part of the writer, that the distinguished author's information must have come from such partial or prejudiced sources as to lead him to somewhat overstate the faults of his neighbors, and to greatly underestimate the really scientific work which is being done in England. R. H. W.

Mr. Zentmayer. It is of convenient model and excellent workmanship, and is combined, very advantageously, with a goniometer attachment represented in the cut by the large graduated circle with vernier. R. H. W.]

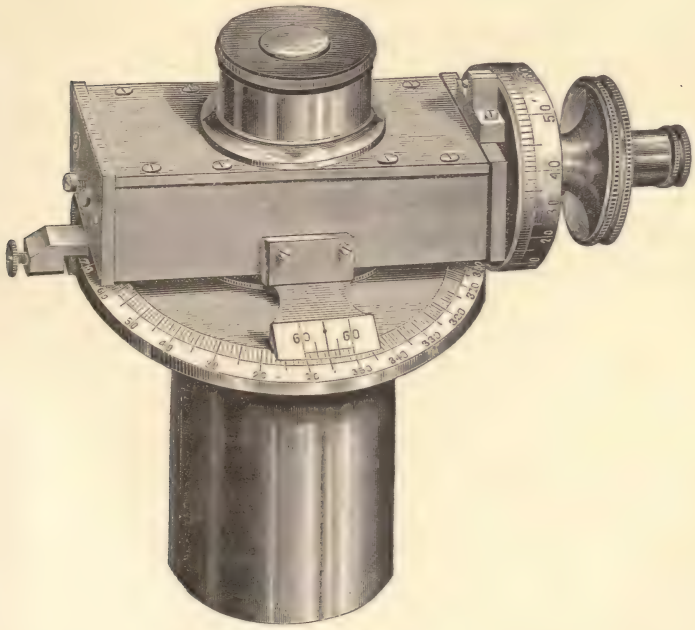


FIG. 54.

III. THE CAMERA LUCIDA AS A MEASURING APPARATUS.

Almost all the contrivances described on pp. 114–118, may be directly applied to the measuring of microscopic objects. If one has traced the exact outline of the object, and knows exactly how many times the image has been magnified, it is only necessary to measure the drawing with the metric rule and divide the same by the number of the magnifications, to get the true size of the object in fractions of a millimeter.

It is first of all necessary, in this case, to determine with exactness the magnification which the camera lucida gives with the objective employed. This may be done in the following way. Set the camera lucida on the microscope and use an objective glass-micrometer for an object on the stage,[3] illuminate it with excentric light so as to bring its markings out clearly, and draw a number of the markings on a piece of paper [at a distance of 10 inches, 25.4 cm., from the eye, and in a plane at right angles, to the line of vision]. This may be done in this way. Draw a straight line with India ink across the paper. Place this line so that it will lie exactly perpendicular to the rulings of the micrometer. Then with a sharp pencil mark the points of intersection of the micrometer rulings with the line, and as the lines by magnification have a considerable size, mark the same edge of each, right or left. The markings in the middle of the field should be selected for this purpose. Then ascertain the distance between any two points on the line by the millimeter scale; repeat this for a considerable number of the markings, and getting the arithmetical average, divide this by the micrometer unit. The quotient gives the number of the magnifications. If one draws an object on paper intended for subsequent measurement, it should naturally be placed at the [standard distance of distinct vision, viz. 10 inches = 25.4 cm. See p. 111].[4]

IV. CONCERNING MICROMETRIC MEASUREMENTS IN GENERAL.

In the descriptions of measuring apparatus we have here and there dropped hints as to their management. Some remarks of a general nature may not be out of place here. Measurements, made by the very best micrometers, only approximately express the absolute size of the measured object. The reason for this

[3] If one has no stage micrometer he may resort to a good eye-piece micrometer, 1 mm. divided into twenty parts and boldly make use of that. It gives, especially when several measurements are combined, perfectly satisfactory results.

[4] If one has a microscope with a draw-tube, he can very easily, with his objective and camera, produce a magnification of any desired round number, which is very handy in simplifying the calculations.

is first of all in the fact that the exact focussing of the object is attended with the very greatest difficulty. Each worker here follows his own subjective judgment, and can follow no other. He works with *his* eyes and *his* hands. When two trained microscopists focus the same object under the same microscope, it may be assumed as a matter of course that the two adjustments will turn out to be different. Indeed, should this not be the case, we must consider it to be purely accidental. That measurements made by each of the two adjustments must differ goes without saying, and therefore it comes about that measurements, made by two different persons, can be compared as to their absolute value only with the greatest difficulty. For this reason all those measurements which are thought to be so extraordinarily accurate, and whose possibility was once so long and so widely discussed, become quite or entirely valueless. But most objects which are to be microscopically measured — organic forms — appear under very unlike dimensions and it must be a matter of great indifference to us whether the long diameter of a grain of starch from a potato, be given $\frac{1}{1000}$ too large or too small.

But micrometric measurements are of particular worth when the results of the same observer are compared for the purpose of obtaining their relative value. But as to getting the absolute results of two different observers, "one may confidently maintain that if the measurements of different observers in any one investigation are comparable, this comparability still continues if the single measure selected were 5 or 10 per cent greater or smaller."[5]

We have still some words to add concerning the designation of the value which we obtain by the micrometer. In former times the line was the unit of micrometric measurement; in France the Paris line; in England the English duodecimal line; in Germany the Paris, Rheinish, or the Vienna line. Since the middle of the present century all these units of measure have been displaced by the millimeter; only the English are so conservative that they still [partially] maintain the line unit, just as in measuring heat they will not exchange the irrational Fahrenheit thermometer for that of Celsius.

On the continent the decimal metric system alone is used.

[5] Nägeli und Schwendener, Das Mikroskop, 1877, p. 285.

The fractional part of a millimeter may be expressed in two ways, by a vulgar, and by a decimal fraction. Hugo v. Mohl[6] earnestly pleads for the use of the common fractions. The designation of micrometric values by the decimal fraction, he holds to be a real nuisance. He thinks that one must be a mnemonic expert to be able to form an intuitive conception of magnitude expressed by a decimal fraction. With Nägeli and Schwendener[7] we are of another opinion. We believe the decimal fractions to be the only logical terms in which to express micrometric values. [Both the advantage and the difficulty of the decimal system are doubtless equally real; but the perplexity which prevents persons not possessed with a mathematical turn of mind from intuitively apprehending the value of fractions extending to several places, especially in decimals, does not apply measurably to a single place of decimals, tenths. Hence the necessity of a minute micrometric unit, especially, but not exclusively, in a decimal scheme. The micron (*infra*) being frequently sufficient without fractions, and never requiring anything beyond tenths except for procedures involving expertness in mathematics, wholly relieves this difficulty, as soon as it becomes familiar to the mind as a unit having a definite value of its own. R. H. W.]

Harting[8] proposed in his time to adopt the one-thousandth part of a millimeter (0.001 mm.) as a unit for giving micrometric values. He would designate this magnitude by 1 mmm., or shorter by 1μ; he named this unit a micromillimeter. This proposition of Harting is doubtless the best and the micromillimeter has maintained itself to this day.[9] If one reads that an object is 23μ long and knows that 1μ corresponds to the unit in the 3d decimal place he can easily construct in his mind the decimal fraction of the millimeter which shall express it (0.023 mm.). But since we usually obtain only the relative size of microscopic objects, so even this latter mental translation is not necessary, for we learn to think of the micromillimeter as a unit, exactly as in common life we do of the centimeter or the mark.

[6] Hugo v. Mohl, *l. c.*, p. 318 *f*—[7] Nägeli und Schwendener, *l. c.*, p. 288.—[8] Harting, Mikr. p. 506.

[9] Listing has also recently pronounced in favor of the micromillimeter as the unit for micrographic measurements. He desires to introduce the name Micron or Micrum for it. (Carl's Repertorium für Experimentalphysik, Bd. x, 1869, p. 5.)

In some exceptional cases, in micrometric measurements, we still have to deal with the old values,—the fractional parts of a line. There are now, for instance, scarcely any objective screw-micrometers made, and those who use this instrument will in most cases have to use an old one, which measures in the fractions of a line. Likewise those statements of micrometer values which occur in works of the first half of the present century are based on the Paris line throughout, so that in order to compare one's own measurements with those, it will be necessary first of all to reduce the latter to the equivalent fractions of a millimeter. I have found, by long use, the tables which follow to be of great practical convenience in making these reductions.

TABLE I.
COMPARISON OF THE UNITS OF MEASURE.

	1 MM. =	1 PARIS LINE =	1 ENGLISH LINE =	1 RHEIN LINE =	1 VIENNA LINE =
Millimeter.	1.0000	2.2558	2.1166	2.1802	2.1952
Paris Line.	0.4433	1.0000	0.9384	0.9964	0.9732
English "	0.4724	1.0659	1.0000	1.0299	1.0371
Rhein "	0.4587	1.0347	0.9710	1.0000	1.0070
Vienna "	0.4555	1.0275	0.9642	0.9930	1.0000

TABLE II.
REDUCTION OF THE UNITS OF MEASURE TO MICROMILLIMETERS.

MICROMILLIMETER	PARIS LINE	ENGLISH LINE	RHEIN LINE	VIENNA LINE
1 μ (0.001 mm.) =	0.000443	0.000472	0.000459	0.000455
2 " = 0.002 mm. =	0.000887	0.000945	0.000917	0.000911
3 " = 0.003 " =	0.001330	0.001417	0.001376	0.001366
4 " = 0.004 " =	0.001773	0.001890	0.001835	0.001822
5 " = 0.005 " =	0.002216	0.002362	0.002293	0.002277
6 " = 0.006 " =	0.002660	0.002834	0.002752	0.002733
7 " = 0.007 " =	0.003103	0.003307	0.003211	0.003188
8 " = 0.008 " =	0.003546	0.003779	0.003670	0.003644
9 " = 0.009 " =	0.003990	0.004252	0.004128	0.004099
10 " = 0.010 " =	0.004433	0.004724	0.004587	0.004555
20 " = 0.020 " =	0.008866	0.009448	0.009174	0.009110
50 " = 0.050 " =	0.022165	0.023620	0.022935	0.022775
100 " = 0.100 " =	0.044330	0.047240	0.045870	0.045550

Table I, "Comparison of the Units of Measure," is in respect to its purpose perfect enough, but the calculations involved in its use are somewhat detailed. There will be necessary in each reduction a multiplication at least, and commonly a multiplication and a division, whereby, on account of the many decimal places, an error might easily creep in. By the use of the logarithm tables an addition and subtraction might be substituted, but the detailed character of the operation would not be altered materially.

EXAMPLE: We find in a work the statement that a microscopic object measures 0.0216 Paris line. We wish to know how much this is in fractions of a millimeter.

$$1 : 2.2558 : : 0.0216 : x$$
$$x = 0.048725 \text{ mm.} = 49\mu$$

Table II, "Reduction of the Units of Measure to Micromillimeters," simplifies the calculations quite essentially. In it is represented the value of the different lines to 1–10, 20, 50, 100 μ

An example will make its use easily understood:

I have found by my micrometer that the length of an object is 28 μ. In an old botanical work I find the same object given as the 0.011841 of a Paris line. I would compare these two values. I find the value of the 28 μ to be in fractional part of the Paris line, according to column 1.

20 μ =	0.008866
8 μ =	0.003546
28 μ =	0.012412
Subtracting the magnitude	0.011841

I get as the difference 0.000571 of a Paris line, which I find, by referring to column 1, is really a difference of something more than 1 μ.

EXAMPLE 2. In an old work I find it stated that the cells of *Nephrocytium Agardhianum* var. *minus*,[10] to be $\frac{1}{200}$ Paris line. I desire to know how great this value is in terms of the fractions of a millimeter. $\frac{1}{200} = 0.005000$ Paris line. The value nearest to that in my table, column 1, is $0.004433 = 10\ \mu$. The difference 0.000567 lies between the values of 1 μ and 2 μ nearest to 1 μ as the table shows it, so that $\frac{1}{200}$ Paris line $= 11\ \mu =$ 0.011 mm.

[10] C. Nägeli, Gattungen einzelliger Algen. Zürich, 1849, p. 80.

Calculations for the English and other lines are made in the same way, using of course the corresponding columns in the table. We have not considered it necessary to give examples for each kind of line.

[The systems in use in micrometry in this country, and in England, are the English and the metric. Of the English inch, vulgar fractions are more commonly used than decimals; partly, perhaps, because so many of those persons, whose education and habits would lead them to choose decimals, are led to adopt the consistent decimal or metric system, to the exclusion of the inch. The use of the line seems to have been abandoned. It can scarcely be doubted that there is a growing disposition to use the metric units in microscopy, the millimeter (approximately $\frac{1}{25}$ inch) for a large unit, and the micromillimeter (approximately $\frac{1}{25000}$ inch) for a small unit; and persons who have once learned to think in these units, so as to avoid the trouble of constant computation, find them most convenient and satisfactory in themselves, to say nothing of the manifest advantages of harmony with the rest of the world. The table opposite, from the Journal of the Royal Microscopical Society, presents in a most available form the relations to each other of the various English and metric units and their fractions. By following, with this table, the directions of the author (p. 132) for the use of his "Table II," observations in inches and those in millimeters or micromillimeters can be transposed with great facility. Moreover the table furnishes the necessary information in regard to metric measures of length, to any who may be as yet familiar with only the English system. R. H. W.]

IV. POLARIZING APPARATUS AND GONIOMETER.

It does not come within the plan of this work to consider critically the process of the polarization of light. Whoever wishes to go into the subject may consult any modern work on physics. For microscopical purposes the subject is developed in the best form by Nägeli and Schwendener in "The Microscope,"[11] and in the work of like name by Dippel,[12] though the

[11] II Auflage (1877) pp. 209–361. [12] Bd. I, p. 224–227, pp. 407–455.

presentation of the matter by the latter is far less satisfactory to me than that by the former.

The microscopical polarizing-apparatus, like every other, comprises an arrangement of two polarizing media between which, in the polarized light, the object to be investigated may be examined. The one placed under the object is called the polarizer and the one over it the analyzer. These conditions in general characterize the positions of the polarizing apparatus of the microscope. The polarizer is most conveniently arranged in connection with the stage. Since it is of a cylindrical form as at present made it may be put into the place of the cylinder diaphragm. Naturally, the analyzer cannot go between the objective and the object, and it is therefore placed either directly over the objective-system or above the ocular. All [Continental] makers now follow Hartnack in adopting the latter arrangement. As a polarizing medium, the Nicol's prism of Iceland spar is the one universally adopted.

As is well known a Nicol's prism consists of two halves; the joining surfaces run diagonally through the prism and form an angle with the end surfaces of 89° 17′. The two halves are cemented together with Canada balsam. The side surfaces are blackened. If a ray of light running parallel to the side surfaces falls upon the lower end of the prism it is separated into two rays, the *ordinary* and the *extraordinary*. The former suffers a total reflection in the layer of Canada balsam and passing to the side of the prism is absorbed in the blackened surface; the latter, on the contrary, passes directly through the prism into the field of view of the microscope which it illuminates.

It is known that in the use of the polarizing apparatus the mutual position of the polarizing media must be changed, and it is all the same if the polarizer be turned about while the analyzer remains fixed, or the analyzer be rotated while the polarizer is fast. One or both must be rotated around its longer axis — the axis parallel with its sides.

The Nicol's prisms, the side edges of which belong to the original rhomboidal calc spar, have a vertical direction, while the artificially ground edges exposed above and below are set

at an angle of 68°. [They are set, by means of a soft cork packing, in brass tubes adapted to the instrument for which they are designed, and the exposed ends are by some makers protected by thin cover-glasses. The polarizer, as mounted, is slipped into the substage ring, and in the better class of instruments can be used in connection with and not in the place of the substage condenser. It nearly always has a rotating movement, and in stands designed for chemical or lithological work a graduated arc by which its position can be known and recorded. It is advantageous to have this prism as large as is consistent with the construction of the stand. The analyzing prism is mounted in a smaller tube. It may be a rather small prism and should be short. It is sometimes mounted in a cap to slide over the top of the ocular, as near as possible to the eye lens, but is more commonly fitted as in Fig. 55 into an

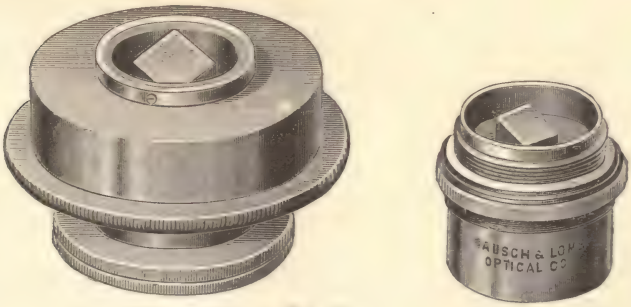


FIG. 55.

adapter with society screw above and below, to be screwed in between the objective and the tube of the microscope. It is frequently so arranged, by cutting away the sides of the containing adapter, that it can be easily rotated when in use. By the addition of a light brass tube, just large enough to contain this apparatus above and to slip over the cap tube of the ocular below, the writer has been able, with great satisfaction, to secure the advantages of both methods of locating the analyzer, which can thus be placed at will in either position. Fig. 55 shows us a common method of mounting the polarizing and analyzing prisms respectively. R. H. W.]

Use of the polarizing apparatus. As is well known the polarizing apparatus is commonly employed to find out if a given object — for example a crystal — has one or two optical axes, if it be singly or doubly refractive. If the crystal be of microscopical minuteness one naturally uses a microscopical polarizing-apparatus. On this account it plays an important role in the hands of the crystallographer. But it is frequently of the greatest use to the botanist. For, in the first place it enables him to know the nature of many of the crystals which occur within the plant-cells, and in the second place all tissue structures are doubly refractive and may be examined with polarized light. Not seldom, details of structure show themselves when they are illuminated thus in the dark field of view which are otherwise not seen at all, or recognized with the greatest difficulty, and thirdly the polarizing apparatus enables the botanist clearly to make out the form of certain microscopic objects.

For an experimental observation with the polarizing apparatus, starch grains from a potato, or a section of the rhizome of *Pteris aquilina*, in which the ducts of the fibro-vascular bundles alone are doubly refractive, will serve us excellently well for an object. A very good object also is a section of the underground stem of *Lathraea squamaria* for in this we have the starch grains and the doubly refracting fibers together.

In the use of the apparatus we proceed as follows. After we have adjusted the object by means of a common ocular and found the best illumination by turning the mirror about, we place the polarizer in position beneath the stage. The field appears to be unchanged, except a slightly weaker illumination. After placing the analyzer also in position, whether above the ocular or above the objective, we rotate the movable part of the apparatus till we bring the two prisms into such a mutual position that the field of vision appears darkest. If the apparatus is well constructed the darkening is almost total. The field appears in a quite deep, very agreeable, shade of blue. With the use of low powers there is a certain amount of light falling upon the object from the side which is reflected upwards, and the consequence is that the field is not perfectly

dark. This difficulty can be remedied by holding the hand so as to shade the object on the stage, or putting round about it for a shade, a piece of angularly folded common blue wrapping paper. The doubly refracting parts of an object are seen on a dark ground, either partly or altogether brightly illuminated, according to their chemical or physical structure. Thus starch grains from the potato appear of a clear bluish shade with a dark cross drawn over them whose radii broaden outwardly toward the circumference.[13] The walls of the ducts from the rhizome of the *Pteris* appear illuminated partly bluish and partly yellowish.[14] As already indicated, we cannot here in the least enter into a discussion of the optical characteristics of organic forms, but must rather recommend the careful study of those parts of Nägeli and Schwendener's as well as of Dippel's work, which deal with this subject.

Rotating selenite plates, which, as is well known, are applied in various ways to the common polarizing apparatus, can be easily added also to the microscopical polarizing-apparatus.

[THE GONIOMETER.]

[For the purpose of measuring the angles of microscopic objects, the goniometer is frequently added to the microscope. This attachment originally consisted of an elaborately mounted ocular in whose field of view was a line which, by rotating the ocular, could be brought successively into coincidence with the different sides of the object, the intervening angles, traversed by the rotating apparatus being read off by means of an attached vernier sliding over a graduated circle clamped to the microscope tube. This accessory may well be combined with the polariscope, as it is frequently used in connection with studies which require polarized light. Such micro-goniometers are still attached to stands designed for chemical or lithological work, but the introduction of the circular concentric stage has rendered them unnecessary except for the use of the specialists. The stand figured in Plate XI, for instance, can be employed for goniom-

[13] The reasons therefor may be found in the before cited works.

[14] For this matter see principally Dippel, *l. c.*

etry without addition or preparation; and any of the circular-stage stands will require for the same purpose only a graduated circle on the edge of the stage. As such graduation is inexpensive, and is also useful for other purposes, it constitutes, for occasional and incidental use, the most eligible goniometer. For the measurement of angles the stage must be carefully centered, and the angle to be measured brought to the centre of the field of view. Such adjustment is facilitated by placing cross lines at the focus of the eye-lens of the ocular as shown in Fig. 56, but only one line *fc* is essential. This line may be either a spider's thread drawn across the center of the opening of the diaphragm of the ocular, or a line ruled on a thin cover glass lying in the same position, one of the lines of an ocular micrometer being often made to answer the purpose. An angle whose plane is parallel to the plane of the stage, and at right angles to the axis of vision, is carefully selected and its apex brought to the center of the field. The line in the ocular is then made to coincide with one of its sides, and the stage is afterward rotated until the other side coincides with the same line, the angle through which the stage and object have moved being readily determined by comparing readings from the stage graduation made before and after the rotation. Should greater precision be required a vernier may be added to give decimals of a degree; but this is not always needed, all measurements with any micro-goniometer being at best but approximate. It is seldom if ever possible to be certain of the exact parallelism of a given plane of the object with that of the stage, and unless that parallelism be secured it is evident that the angle will be seen in perspective and will be incorrectly stated in the reading of the instrument.]

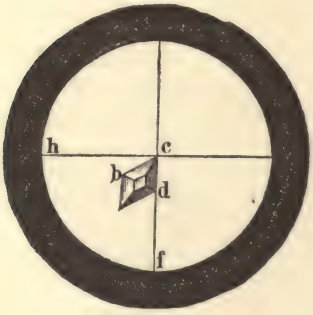


FIG. 56.

[Mr. Zentmayer combines a goniometer attachment with his cobweb micrometer as shown in Fig. 54, the lower graduated circle being firmly attached to the microscope body, and the ver-

nier above it enabling us to read off accurately the extent to which the micrometer with its field crossed by fine lines has been rotated. A pair of extra lines, crossing each other in the center, are provided for the accurate centering of the angle to be measured. The analyzing prism, when required with this combination, is placed above the objective. R. H. W.]

V. THE MICRO-SPECTROSCOPE.

In recent times investigations are frequently carried on by means of a spectroscopic apparatus combined with the microscope. The spectro-microscopical apparatus, especially in the hands of botanists, has become an important instrument in the investigation of the coloring matter of plants. Since we have found an adequate description of the micro-spectroscopic apparatus[15] in no existing work, we shall here attempt to consider it somewhat in detail. We shall found our description on that most perfect construction of it which was first given to it by Sorby and Browning.

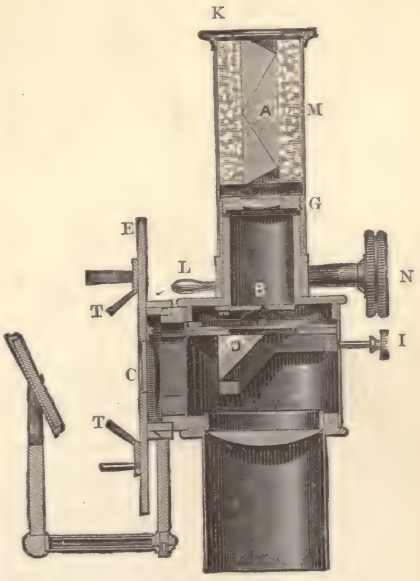


FIG. 57.

A. The Prisms. It is well known that when a ray of so-called white light passes through a massive glass prism, provided that the refracting edge of the prism be perpendicular to the ray of light, it will be separated into its elementary colors. Then there is produced a spectrum (solar spectrum) whose colors and their arrangement are well known. Now, if in place of the dispersing prism we substitute a combination of three or five prisms of crown and flint glass alternately, arranged as is shown in Fig. 57 at *A*, the light will pass through

[15] Nägeli and Schwendener, *l. c.*, p. 39.—Frey, Das Mikroskop, p. 36, *f.*

it in a direction parallel to its axis. This arrangement is here known as a direct[16] vision spectroscope.

The prisms are fastened into a brass cylinder *M*, by means of a cork mounting. The cylinder is closed above by a dull black metal plate *K*, with a round hole in the middle about 10 mm. wide for looking into. This apparatus is connected with the ocular and is placed above the eye-lens *G*. Should the ocular have a common circular diaphragm, the field of the microscope, after putting the spectroscopic apparatus in place, should appear to be a small ellipse, the center of which should be quite colorless, and at the points of greatest curvature red and blue respectively. Microscopic objects which do not fill the field seem to be striated in the direction of the longer diameter of the ellipse. If in place of the diaphragm-opening of the spectroscopic ocular we substitute a slit-opening which is so arranged in respect to the prisms that the refracting edge is parallel to the slit *B*, a spectrum will now appear of the well-known band-form whose brightness and extension are conditioned by the breadth and length of the slit.

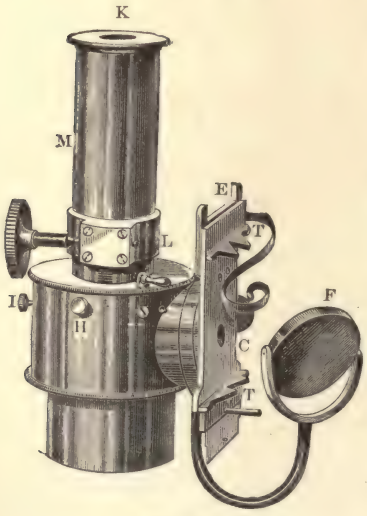


FIG. 58.

B. The Slit. To constitute a practical working instrument the slit must be exactly adjustable in the focus of the eye-lens of the ocular, for every eye, and it must be so contrived that the slit may be narrowed or widened, lengthened or shortened at will.

The focussing of the slit is easily provided for by having the tube of the ocular made of two parts, one shoving into the other and moved by a rack and pinion.

[The contrivances for widening the slit are about as various

[16] This combination was first contrived by Amici in 1803, later applied to the construction of a pocket spectroscope by Hofman (see Schellen, Spectralanalyse, p. 109) and finally proposed for the microscope by John Browning.

as the ideas of the different makers. It is only essential that the two shutters forming the sides of the slit should steadily approach and finally meet each other in the center of the field, with a perfectly smooth and parallel motion, which is under complete and easy control. In Fig. 57 this motion is controlled by a small milled head. Shutters moved by the lever L are provided for controlling the length of the slit. R. H. W.]

C. The Comparison Prism. It is often very desirable to compare the spectrum of a body which is being investigated with that of a like body. This may be done by first observing the spectrum of the body under investigation, and after that the spectrum of the body used for comparison. But the spec-

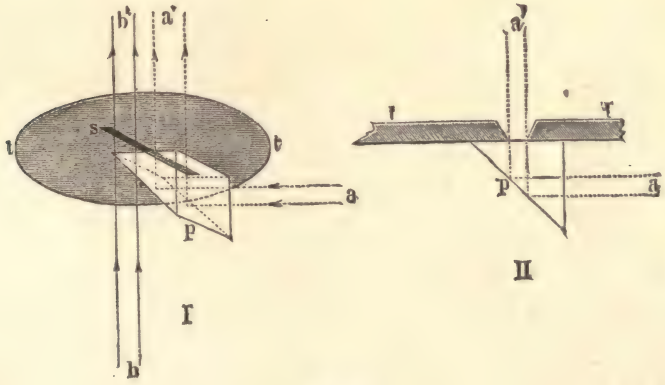


FIG. 59.

tra, for example the absorption spectra of some colored fluids with many dark bands, are found to be very difficult to carry in the memory so as to make the comparison. Hence a comparison of the spectra in this way can relate only to the leading features and not to smaller particulars. But in order to make comparison of the finer details with sufficient leisure and exactness we must see both spectra very near together, and at the same time — the so-called double spectrum. This we can do by means of the comparison prism, an apparatus for which we are indebted to Kirchhoff.[17]

Suppose *tt*, Fig. 59, I, be the plate of the drum which bears the

[17] Schelling, Spectrum analysis in its application to the substance of the earth and the nature of the heavenly bodies, Brunswick, 1870, p. 121.

slit *s*. The body to be investigated is at *b*. The rays from it pass in the direction *bb'* through the slit and at *b'* enter the combination of refracting prisms. The slit is not opened along its whole length for the admission of rays from *b*. For half of its length it is covered by a reflecting prism of the form which we have come to know in the apparatus for microscopical drawing (see p. 114, Fig. 45). The prism in section represents a right angled isosceles triangle, Fig. 59, II, the hypothenuse surface being inclined to the slit at an angle of 45°. Now, if we suppose another body to be used for comparison placed so as to send rays from *a* in a horizontal direction, they fall upon the surface of the prism perpendicularly, and unrefracted pass to the hypothenuse surface and fall upon it at an angle of 45°. Here they are totally refracted perpendicularly upwards, and as *a'* pass through the slit parallel with the rays *bb'*. If now the refracting prisms are placed over the slit as already described the observer will now perceive not one but two spectra lying next each other whose colors, Fraunhofer lines, etc., exactly coincide. We call these two a double spectrum.

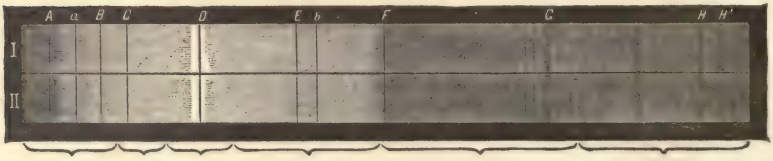


FIG. 60.

Fig. 60 represents a double spectrum which is produced by the rays coming through the free half of the slit, and by those of diffused daylight passing through the comparison prism. I is the spectrum of the rays through the prism, II that of those not reflected. They are separated by a slender black line which one commonly notices but which when one has the eye close over the refracting prism has no disturbing influence. We notice in the spectra the strong Fraunhofer lines; those of the under spectrum falling exactly in the prolongation of those of the upper. [The comparison prism, not being required for

constant use, is mounted in a sliding frame and can be instantly slipped into or out of the field of view by means of the milled head I, Fig. 57. R. H. W.]

Laterally, on the largest tube or drum, is a perpendicularly arranged plate *E*, Fig. 57, provided with spring clamps, which has the form of a microscope stage before which may be seen a plane mirror *F*. The plate has a small opening in the middle at *C* which should be closed when the comparison plate is not in use. If one wishes to use the comparison prism he pushes the milled head *I* forward and the prism which has been lying in the side of the drum is shoved into place over one-half of the slit. By proper adjustment of the mirror the necessary light can be directed through the opening *C* in the stage *E* upon the comparison prism. For the production of the comparison spectrum we should bring the comparing body as closely as possible before opening in the plate *E*.

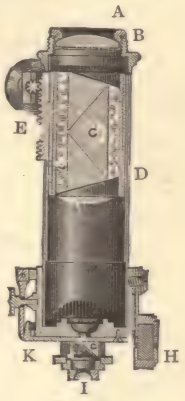


FIG. 61.

[*D. The Binocular Micro-Spectroscope.* The spectroscopic ocular hitherto mentioned is the original form contrived by Mr. H. C. Sorby of London and elaborated by John Browning a distinguished manufacturing optician of that city. It is still the form in most general use. It is shown in Fig. 58 complete and ready to be inserted in any microscope in place of the usual ocular. Mr. Sorby has more recently contrived another form known as the Binocular spectroscope. In this arrangement, Fig. 62, and shown in section in Fig. 61, the prisms are transferred from above the ocular to below the objective, giving greater dispersion as well as fitness for binocular use. The apparatus screws into the tube of the microscope, in place of the objective by the screw *B*. It consists of a small collecting lens *I* which when in use should just touch the object under investigation; a slit adjustable by the screw head *L* and a set of prisms *c* through which light from *I* passes upward to the objective *A*

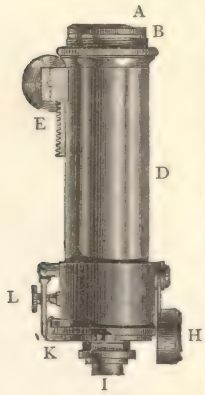


FIG. 62.

and thence through the microscope tube and ocular. The comparison prism *G* can be made to send up either the absorption bands of a known solution or the interference spectrum from the standard scale *H*. This apparatus, like the preceding, is of the Beck style, supplied in this country by Wm. H. Walmsley and Co. of Philadelphia. R. H. W.]

E. The Measuring Apparatus of the Micro-spectroscope. It is known that Bunsen and Kirchhoff, in their chemical investigations upon the absorption spectra and the Fraunhofer lines, divided the whole length of the spectrum into 170 equal parts, and determined the position of the Fraunhofer lines in the solar

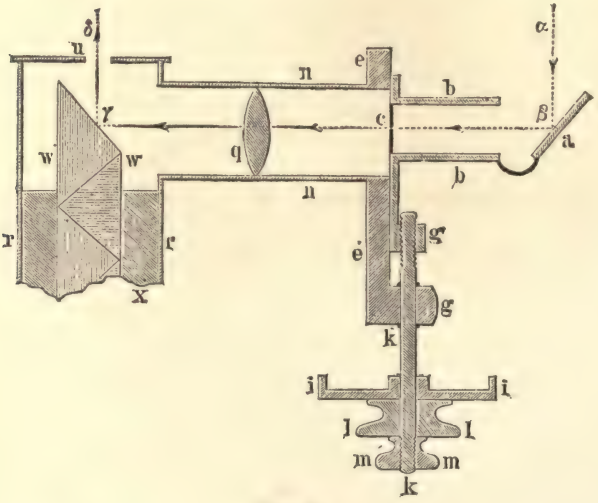


FIG. 63.

spectrum and the illuminated bands in the discontinuous spectrum according to a scale. In general this scale is fundamental in spectrum analysis. The common spectroscope for the purpose of spectrum analysis has a tube, in the front end of which is a glass on which is photographed a millimeter scale reduced about fifteen times. It can be illuminated by light admitted for the purpose. The image of this scale is thrown by means of a biconvex lens on the front surface of the refracting prism, this is reflected into the observing telescope and reaches the eye of the observer as an optical image apparently lying on the

spectrum. The contrivance, however, suffers from this fault that the thickness and brightness of the division lines are dependent on the width of the slit, which is different with different eyes. But when we undertake to measure the exact distance of the Fraunhofer lines and the breadth of the absorption bands we have to give up this very simple and practical contrivance.

The idea of an exact measuring apparatus for the micro-spectroscope, which we may call really ingenious, originated with Mr. John Browning of London. This not very simple contrivance is illustrated in section in Fig. 63. First is *rr* the perpendicular brass tube which incloses the five prisms *ww*, in their cork mounting *xx*. The upper surface of the prism $w\gamma$ is inclined to the horizon at an angle of 45°. Opposite to it is the horizontal tube *nn*, provided within with a biconvex lens *q*, which is movable in the direction of the length of the tube *nn*. This tube *nn* bears upon its front fastened to it the brass plate *ee*.

FIG. 64.

In a dovetail guide on this (movable longitudinally) is the plate *g* on which a narrow brass tube *bb* is securely fastened. The latter is closed at *c* with a tinfoil plate in the middle of which is made a very small opening of the form of an equilateral triangle. Outside, *bb* carries a double arm on which the small, movable, plane mirror *a* is held. Now, by illuminating the triangle at *c*, by means of the mirror *a* and giving the lens *q* the right adjustment for the eye of the observer, we shall see, by bringing the eye to the point δ, a white illuminated, magnified image of the triangle. The course of the rays and their double reflection are indicated by the dotted line $\alpha\beta\gamma\delta$. If, now we suffer the light to pass through the prisms *ww*, we shall perceive a spectrum on which the triangle seems to be lying. By a suitable adjustment of the whole apparatus it can be brought about so that the image of the triangle will appear within the spectrum or upon its upper or lower border. Fig. 64, for

example, shows a single solar spectrum on the upper edge of which this measuring triangle lies.

In order that this ingenious contrivance may be useful in measuring distances on the spectrum, the triangle must be movable along the length of the spectrum and the amount of the movement must be capable of the most exact measurement. We must keep in mind, however, that all determinations of length in the spectrum represent only relative size. There is no such thing here as determining absolute magnitudes.

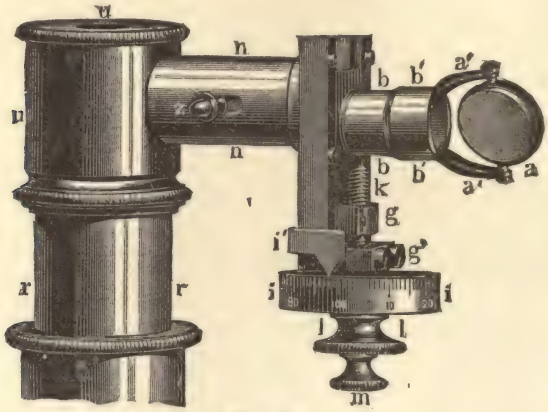


FIG. 65.

The contrivance which serves our purpose in this is quite the same as the one already described in the objective screw-micrometer. It is diagrammatically sketched in Fig. 63. The plate *ee* carries in a brass projection g' a micrometer-screw *k* which rotates but does not progress. The thread of the screw works in a like projection of the plate which carries the triangle *c*. By turning the screw this is moved up or down in a dovetailed bearing or guide on *ee*. The amount of this movement which corresponds to the size of the screw thread may be read off on rulings engraved upon the inner plate and playing by an index on the outer; fractions of the same may be read from the drum *ii* whose periphery is divided into 100 parts.

We shall now understand the illustration, Fig. 65, which represents the whole measuring apparatus in about $\frac{7}{8}$ its natural size. The letters *a b k g g' i l m n u* and *r* are of like signifi-

cance with the corresponding ones in Fig. 63. The two metal arms on which the mirror is mounted and rotates are represented by $a'\,a'$. They spring from the brass ring $b'\,b'$ which rotates on $b\,b$. At i' we see the index by which the drum with its rulings passes, and z is a small handle by means of which the lens q is moved and the image of the triangle adjusted. Naturally, the tube u must fit very exactly upon $r\,r$ which holds the prisms, or the measuring would be very inaccurate.

In order to measure by means of this contrivance, the spectroscope is taken off the microscope tube, and the measuring apparatus carefully adjusted and then replaced. The little mirror on the transverse tube must be so arranged that the light will fall upon the triangle and then the lens should be adjusted by means of the little knob in the tube. To obviate any parallactic displacement during the measuring, this adjustment must be such that it and the Fraunhofer lines adjusted by means of the pinion N, Fig. 57, come to exactly the same visual distance. We may know when this two-sided adjustment is exactly right by the fact that a movement of the head from side to side does not show the triangle, which has been placed on a given line, moving across the lines. If the apparatus is made with precision it will allow of measurements of extraordinary exactness. We should not remove the apparatus from the microscope till after the measuring is finished for the removal and replacing of the instrument might easily make a difference of several divisions of the drum head in the distances determined.

Each observer, before he can use the instrument in investigations, must provide himself with a scale (which for eyes of different visual lengths will be different), in which the position of the several Fraunhofer lines are exactly designated. This, once constructed, serves as the foundation of all measurements. It will commonly be sufficient when we have determined the distance of the Fraunhofer lines A to H or H' (see Fig. 72, p. 153) in divisions of the measuring[18] apparatus and with this unit the distance apart of the lines A, a, B, C, D, E, F, G, as found by measuring. If by subsequent measurements one

[18] With the apparatus (Seibert and Kraft) which I have used and my eye, the distance from A to H is 752 graduations of the drum.

would eliminate every possible error he should, after the completion of such a scale, focus the triangle on a given Fraunhofer line and then see if it lies exactly on the point designated in the scale. A casual difference may arise in the calculation.

Example for the Application of the Measuring Apparatus.

We here add a concrete example to illustrate what has just been said.

It is required to measure the relative distance of the Fraunhofer lines *D*, *E*, *b*, *F*.

We focus the triangle on the upper edge of *D* and notice the position = 223.3. Then turn the micrometer-screw forward to *E* and read off = 362.5. Distance then from *D* to *E* = 139.2. Now turn forward to *b* = 382.5. Distance from *E* to *b* = 20. Finally turn to *F*, read = 490.7. Distance from *b* to *F*, = 108.2. According to a lithograph spectrum table of Bunsen and Kirchhoff founded on the scale of 170 divisions (see p. 144) there is given *D* to *E* = 21.2, *b* to *F* = 15.7.

Comparing the distances *D E*, and *b F*, according to our measurement $\frac{D\,E}{b\,F} = \frac{139.2}{108.2} = 1.2865$

Comparing the same distances $\frac{D\,E}{b\,F} = \frac{21.7}{15.7} = 1.293$ accordimg to Bunsen's table.

Difference of the two values 0.0065.

[*The Standard Scale*, also devised by Mr. Sorby, is a simple and convenient means of measuring the position of absorption bands, when great precision is not required. It consists of a plate of quartz cut parallel to the principal axis of the crystal to the thickness of about 1.09 mm., and mounted between two Nicol's prisms or Herapathites. When light is transmitted through this combination, it is evident, from the well-known property of quartz under polarized light, that an interference spectrum will be formed. This spectrum is divided into twelve optically equal parts by black bands, the third space counting from the red end towards the blue corresponding exactly to the position of the *D* or sodium line. The optical parts are enclosed in a tube attached to a plate, Fig. 66, which can be placed at *c* of

Fig. 57; so that light from the mirror *F* will pass through the combination before entering *c* and will be reflected upward by the comparison prism *D* to the eye at *K*. The half of the field of view which pertains to the comparison prism will be occupied therefore by an image of the standard scale, with its twelve well marked divisions, while the other half of the field will show in direct relation to this the spectrum bands of the object on the stage of the microscope. The standard scale *H* shown *in situ* upon the binocular spectroscope, Figs. 61 and 62, is a very compact form in which the Nicol's prisms are replaced with thin plates of iodide of disulphate of quinine known as Herapathite. Fig. 67 shows the appearance of a field of view in which two absorption bands in the upper half of the field are being located by means of the image of the standard scale in the lower half. R. H. W.]

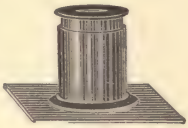


FIG. 66.

F. Using the Micro-spectroscope. To the botanist the micro-spectroscope is an apparatus of such importance that it indeed must be occasionally used by every one who shall undertake the thorough microscopical investigation of plants. A few remarks therefore concerning its use will not be superfluous.

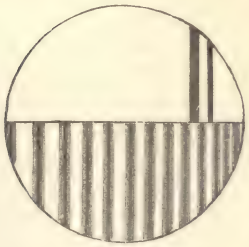


FIG. 67.

The objects to be investigated are of two sorts, liquid and solid. Coloring substances as chlorophyll, phykophaein, etc., will frequently come under micro-spectroscopic investigation in the form of a solution. In general we need scarcely say anything concerning the preparation of the solution. In reference to the chlorophyll of the phanerogams especially, the particular part of the plant from which the preparation is to be made, as for instance the foliage leaves, is put for a short time in boiling water, then quickly dried by means of bibulous paper and then immersed for a longer time in absolute alcohol, ether or benzole, in a dark place, for the purpose of extracting the chlorophyll coloring matter. The concentration of the solution thus produced, which influences the intensity of the absorption spectrum and the number and length of the absorption

bands, depends naturally upon the time during which the material is in the extracting medium, as well as on the quantity of the material. Commonly also a solution of less concentration will give the same intensity of spectrum if a sufficiently thick layer of it be used. The solution can commonly be examined in an ordinary test tube. The test tube is filled and carefully corked and then laid on the stage of the microscope, or held before the opening of the comparison prism as the case may be. For the latter purpose (bringing liquids before the opening of the comparison prism), a small open trough, made of zinc, to which are cemented two parallel glass plates, as shown in Fig. 68, is very useful. For exact investigations, however, the trough flask illustrated in Fig. 69 is preferable. It is a flask, whose two sides, back and front, are parallel, furnished with a carefully fitted ground-glass stopper. It should be filled quite full of the solution and then laid with its broad side on the stage. It is especially indispensable when we wish to study the combination spectrum of two solutions. In that case two flasks are filled each with a different solution and both laid upon the stage one upon the other.

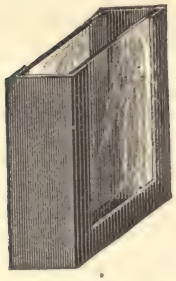


FIG. 68.

FIG. 69.

[For the purpose of examining small quantities of any liquid, a sufficient depth being obtained with very little material, vertical glass tubes attached to horizontal plates are used, as proposed by Mr. Sorby and shown in Fig. 70. The narrow tubes are made of various lengths in order to present different thicknesses of the contained fluid; the broad tube being higher on one side than the other and thus constituting a wedge-shaped cell, which

when filled and closed by a thin cover-glass will present a varying thickness of fluid for study and comparison. R. H. W.]

If the object to be investigated is not a solution but a preparation of the kind which we commonly employ in microscopical inquiries, we must first of all bring it into the focus of the objective-system. To do this we must first remove the tube bearing the prisms, open the slit somewhat and use the apparatus as a simple ocular.[19] If one has to deal with a small object which would not entirely fill the slit, but so that rays of light might come in by it and disturb the spectrum, he should turn the comparison prism so as to shut up some of the slit without, however, letting in the light upon it, and then bring the object up near to it and from the other side shove up the shortening apparatus as close as is necessary. On the other hand,

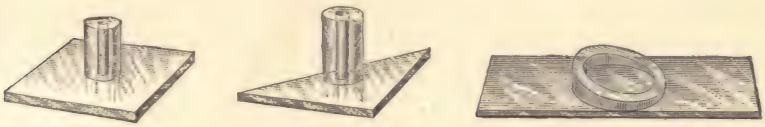


FIG. 70.

should the object consist of a number of single minute grains, which would cause to be drawn across the spectrum, in the direction of its length, perpendicular to the Fraunhofer lines, a like number of dark lines, one must adjust the microscope so that the object will be a little out of focus, somewhat above or below the true focus. In this way we shall get a uniform spectrum.[20] [The spectrum can also be improved in some other cases by likewise throwing the object somewhat out of focus. R.H.W.]

The Influence of the Slit on the Spectrum. In the investigation of homogeneous solutions it is by no means a matter of indifference what width we give to the slit, as will be clear from the following considerations. *S S*, Fig. 71, is the slit of a spectro-microscope, *P* the refracting prism, and *W* a screen which will intercept the spectrum produced by *P*. The slit *S S* shall be wide enough to afford entrance to the three rays of white

[19] Be careful not to open it too wide, else in closing it again one might easily get dirt on its edges.

[20] See G. Kraus, Zur Kenntniss der Chlorophyll farbstoffe, Stuttgart, 1872, p. 12, *f.*

light *a b c*. The ray *a* will be separated into its elements by *P* and produce the spectrum *a'* at *W*. Likewise *b* produces *b'* and *c* the spectrum *c'*. The three spectra in their middle parts, green and yellow, overlap each other and produce here a great number of mixed colors. But had the slit been narrowed by the distance *c b* then *c* would not have entered and the spectrum *c'* would not have been produced, and the number of mixed color in the middle would have been also less. Therefore the narrower the slit, the purer and richer will be the colors of the spectrum. By opening the slit wider the spectrum will indeed be brighter, but it will be pure only at the red and blue ends while in the middle there will be a prevalence of compound mixed light from all the possible beams, and here the Fraunhofer lines cannot be seen.

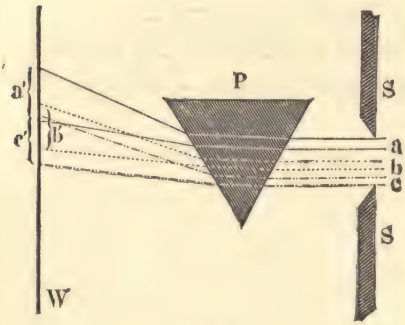


FIG. 71.

It is of the first importance not only to devote attention to the width of the slit, but also to keep the edges of it absolutely clean. For if the smallest particles of dust get upon them they will show themselves in the spectrum by a like number of black lines drawn over its whole length which very greatly disturb the observation. As we have explained, a pinion with a milled head serves to adjust the ocular lens to the slit and the Fraunhofer lines. The adjustment differs with different eyes. By turning the milled head back and forth, the ocular is so placed that the slit will appear sharp, and when daylight is used, the Fraunhofer lines will appear with the greatest possible sharpness and distinctness.

If the apparatus is a good one a great number of these lines may be seen. They furnish the best test of the performing qualities of the apparatus. We give, in Fig. 72, an illustration of a spectrum made from common daylight by means of Seibert's spectroscope into which the visible Fraunhofer lines between *D* and *F* are brought. Their distances apart have been

determined by the measuring apparatus constructed by the same firm.

Suppose now we had for examination the spectrum of some matter, for example of a kind of chlorophyll from the leaves of *Primula* (after Kraus). We should observe in this spectrum:

(1) The number of the absorption bands.

(2) Their position and breadth.

(3) Their relative brightness.

FIG. 72.

The number of the absorption bands can evidently be found by counting. Their position can be determined in two ways. First, one can determine this approximately by fixing in the eye the Fraunhofer lines and estimating the position of the bands in relation to them. The breadth may be obtained in the same way by estimating their distance from two lines in the neighborhood. But this method will yield only approximate results.

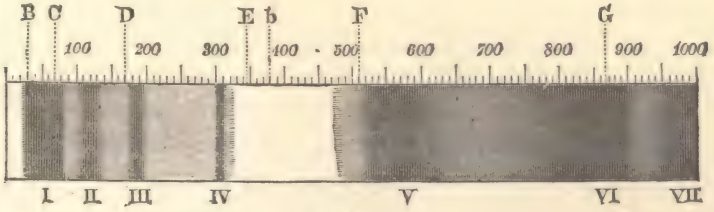


FIG. 73.

If one would be exact he must have recourse to the measuring apparatus. The measuring is done by moving the triangle over the whole length of the spectrum and noting at the beginning and end of each band the position of the index. It is much more difficult to estimate the relative brightness of the absorption bands since here we bring to bear a subjective judgment only. In the case before us we come to the conclusion that the absorption bands, *B C* (Band I, Fig. 73) and *F* (Band V) are

the darkest, the spectrum colors are at these points most perfectly absorbed. The bands II, VI and VII are of medium brightness, while the two bands III and IV are the brightest of all. There are no other mechanical aids for these determinations at the present time. A micro-spectro photometer is not known to us.

Since measuring the breadth and position of the absorption bands often consumes much time, the comparison of spectra may be employed with advantage, when the spectrum of the object being investigated seems to be like that of one already known to us. We put the material under investigation (we will suppose a solution) in a trough on the stage, and the known solution before the opening of the comparison prism, and shove the prism into place. If then the position and breadth

FIG. 74.

of the bands are alike it is sufficient grounds for assuming the solutions to be identical. If the material is not identical but related, then the differences in the spectra will be small, and can be easily indicated on paper by means of the measuring apparatus. We see illustrated, for example, in Fig. 74, I, the well known spectrum of oxyhämoglobin. With this is the spectrum II, of a substance (hämoglobin, reduced hämoglobin) for comparison, which is produced by giving to the former solution some drops of the solution of ammoniacal iron tartrate.

We will consider here only the absorption bands between *D* and *E*. We need not measure the darkest part of the absorption band of II since it lies exactly between the two dark bands of the comparison spectrum. This is necessary only for the outer borders of the brighter absorption stripes. For this purpose the measuring triangle must be focussed on the dark line

which separates the spectra and then determine the distance from the edges of the absorption stripes in II to the nearest lying edges of those in I, Fig. 74.

Should we wish to examine the spectra of the same fluid in layers of different thicknesses, we might put it into long glass tubes which have been melted off even at the bottom, and which may be shoved into the tube of the microscope when the objective has been removed, according to Pringsheim's method. Then by gradually filling the tube with the solution one can easily observe the changes which take place in the spectrum.[21]

[21] For the technique of micro-spectroscopical investigations one may consult: G. Kraus, Zur Kenntniss der Chlorophyllfarbstoffe, Stuttgart, 1872. Pringsheim. Ueber die Absorptionspectra der Chlorophyllfarbstoffe (Monatsber. der K. Academie der Wissenschaften, zu Berlin, Oct., 1874, pp. 628-659, nebst 1 Tfl.).—Nägeli und Schwendener, Das Mikroskop, pp. 436-440.

CHAPTER III.

THE PREPARATION OF MICROSCOPICAL OBJECTS.

I. INTRODUCTION.

Although there are many opportunities to buy ready made microscopical preparations, still every one who uses the microscope for something more than amusement is compelled so to prepare those parts of plants which he is to examine that they will be suitable for microscopical research. And it will often be desirable to prepare the object to be investigated so that it may be preserved for any length of time, and placed at any moment under the microscope. There has therefore sprung up, chiefly in recent times, a microscopical *technique*, to learn which indeed requires a pretty long schooling, but which may be learned by any one if he does not lack the necessary time and perseverance. For those especially who have no patience, the preparation of microscopical objects is the best means of attaining it. But those who have patience will often enough lose it in this employment. One must not be afraid of making failures at first, even though they should be repeated many times, but remember that perseverance always leads on to success.

We remarked that microscopical technique had been built up in recent times. Formerly it was thought that if a part of a plant needed anything to be done to it before putting it under the microscope, it was sufficient simply to crush it. Sachs has mentioned in his "History of Botany"[1] the sad influence, upon the development of vegetable anatomy, of this rough method of preparation which prevailed during the whole of the last century. It was in the first third of our century that Hugo v. Mohl undertook to bring the preparation of microscopic objects to the excellence which they have reached in our time.

[1] Page 265, *ff.*

He first emphasized the advantage of immersing every object which was to be submitted to microscopical investigation, as far as possible, in some fluid. He first introduced the cover-glass into general use.

Of almost still more recent date is the preparation of those objects which are to be preserved for a long time and are called *permanent preparations*. Who has not seen lying by old microscopes wooden strips with a series of holes in them, in which two round glasses were clamped by means of an elastic metal ring, bearing between them the preparations (1) a fly's wing, (2) a leaf of moss, (3) a human hair, (4) a spider's foot, (5) a linen thread, (6) the best of all, a flea?[2] How lamentable was the case in respect to the preparation of permanent mounts even down to Hugo v. Mohl's time, one may best get an idea by reading through the conclusion of his "Mikrographie" of 1846.

He proposed that the object, in case of a dry preparation, should be placed between two slips of mirror glass (of the size of our slides) and in order to keep the dust out to paste the edges about with strips of paper. If the object were to be preserved in a moist condition, a drop of the solution of chlorate of lime was placed between the glasses, the object immersed in it, and inclosed by two paper slips smeared with a thick rubber gum solution and put across between the glasses. No one nowadays thinks of preserving objects in this way. We shall see presently that we have now other and better methods of preparation.

*

* *

It is necessary, in the first place, to know exactly how a microscopical preparation should be made so as to satisfy all requirements.

The first, and in all cases the same requirement, is that the part of the plant under consideration shall be by nature, or shall be made, so thin that light may go unhindered through it, so

[2] In the catalogue of Leeuwenhoek's collection of microscopical preparations, which has been preserved, occur, "a hair from the nose," and "oysters not yet hatched out in a small tube."

[3] P. 328, *ff.*

that it shall be perfectly transparent. The examination of opaque objects by reflected light does not occur so far as I know in vegetable anatomy. The preparation should also exhibit the parts investigated in their natural and undisturbed condition. Objects which indeed are thin enough, but whose individual parts are separated from each other and torn, are commonly not in a condition for satisfactory examination. Finally, objects must be examined in a medium (fluid), which will allow the structure to be seen in the most natural possible condition. I say in the most natural *possible* condition because there is no fluid known perhaps which will show the microscopic object altogether naturally.

The fluid applied to the object may impart fluid to it, or withdraw fluid from it. In the first case the organ will swell and in the second it will shrink. If, for example, one puts chlorophyll or starch grains into a solution of calcium chlorate, they will immediately swell, while cell walls remain quite unchanged in it, but the latter immediately swell if they be put into a concentrated solution of chlor-iodide of zinc. Potassium bichromate and chromic acid, also alcohol and glycerine, often produce a shrinking, and the latter always contracts the primordial article. One might perhaps think that water would act indifferently upon the preparation, but this is by no means the case. Gum arabic immediately swells up in water and becomes a mucilage, likewise all muciparous cell walls are thus immediately changed in this medium, by considerable swelling. One must in each case choose the right medium for the investigation and will get, for example, a much more natural image of the muciparous cell walls in absolute alcohol than in water.

It is well known that fluids possess a much greater refractive power than the air. We express the magnitude of this by means of the *refractive index* or the *exponent* of *refraction*, that is to say, in the passage of light from the air into a given fluid, there is a constant relation between the sine of the angle of incidence and the sine of the angle of refraction. The numerical expression of this relation is the refractive index or exponent. Thus the exponent of refraction of some of the more important fluids, suitable for microscopical use, is as follows:

Anise oil,	1.811	Sulphuric acid,	1.430
Tolu-balsam,	1.628	Glycerine and water	
Cassia oil,	1.610	(equal parts),	1.400
Canada balsam,	1.530	Glacial acetic acid,	1.380
Citron oil,	1.527	Alcohol,	1.370
Turpentine oil,	1.476	Ether,	1.360
Pure glycerine,	1.475	Albumen,	1.350
Olive oil,	1.470	Water,	1.336

It is now a well-known fact that an object becomes more distinctly visible, the more its refractive power differs from that of the medium in which it is mounted. We have already seen that it is not a matter of indifference how a specimen is mounted which is to be brought under observation, as in the case of the test objects (butterflies'-scales, diatoms, etc.), whether mounted dry and so surrounded by air only, or lying in Canada balsam. In the former case their delicate, interesting markings are much more easily seen than in the latter. Very delicate plant structure is much more difficult to recognize in glycerine than in water. It is therefore recommended that the fluids used in preparing objects for observation be such as have the least possible refractive power.

But, if for any reason, the object be too opaque for microscopical observation, then the greater refractive power of the fluid serves as an important means for rendering it transparent, and brings to view details which in water or fluids of like refractive exponent would not be seen. The beginner will most easily see the clarifying power of fluids of different refractive index if he will examine the large pollen grains (of *Epilobium*, *Cucurbeta*, *Malva*, etc.), first in alcohol, then in Canada balsam and in anise, or clove oil. If in practice one wishes to find a fluid of most suitable clarifying powers for a preparation, it may be found most conveniently in the table just now given.

How very much the refractive power of the mounting fluid determines the visibility of a microscopic object is illustrated by this experiment. A fine glass rod lying in water is very easily recognized by reason of the difference of their exponents of refraction. But if we lay it in Canada balsam, the exponent of which is very nearly the same as that of the rod, the rod will

cease to glitter and it will be only by giving the closest attention that we can distinguish it, and then as a flat band. But if it be put in anise oil we get an image of it as if it were a a hollow space run through the oil. (Welcker.)[4]

II. THE PREPARATION OF OBJECTS WITHOUT CUTTING INSTRUMENTS.

Except in rare cases microscopic objects will be obtained by dissecting out the parts of the plant with a knife. To these exceptional cases, however, belong all such plants and plant organs as are so thin that they may be laid under the microscope without further manipulation, and also those fragments of thicker parts which one may obtain by maceration or incineration.

A. Objects for Immediate Observation.

As such, we name first the delicate hairs of the higher plants, next those parts of the cuticle which are easily torn away, then those leaves of mosses and liverworts which consist of a single layer of cells, and finally a great number of algæ and fungi.

Delicate hairs only need to be separated from the plant by means of a sharp knife, or torn off with sharp forceps and put in water or glycerine to be immediately subjected to investigation. In this way, for instance, we obtain the beautiful preparation which shows the circulation of the protoplasm in the staminate hairs of *Tradescantia*, or in the root hairs of *Hydrocharis Morsus ranæ*. Likewise in this way, a piece of the epidermis of higher plants may be immediately examined which one may tear away from the leaves with the forceps. The leaves of many monocotyledons (*Lucojum*, *Galanthus*, *Hyacinthus*, *Orchis*), lend themselves to this procedure most readily. The leaves of mosses and liverworts may be broken from the mother plant with the forceps and immediately immersed in a drop of water and laid under the microscope.

[4] Frey, Das Mikroskop, Leipzig, 1877, p. 73.

A great number of the lower thallophytes may be subjected to examination without previous preparation. Of the many-celled plants belonging to this group, are the Hydrodictyeæ, Ulotrichaceæ, Zygmaceæ, Mucorineæ, Piptocephalideæ, Sphaeropleæ, Oedogoniaceæ, Confervaceæ, many Ulvaceæ, Coleochæteæ, and many others, and the great number of the single-celled thallophytes including all the swarm spores.

The single-celled organisms, on account of their almost or quite microscopical minuteness, are very difficult to bring into the examining drop under the microscope; in many cases it is accomplished only by a fortunate accident. The larger of them however may be best found by the following simple means first suggested by Ehrenberg. Fill a wide watch-glass with a portion of the water which we suppose contains the larger forms of the single-celled algæ, and place it on a piece of white paper which has had one-half blackened with India ink, so that the background of one-half of the glass is black and the other half white. On the dark background the light colored and on the white the dark colored plants become conspicuous. Examine the glass now with a not too weak magnifier and we shall easily distinguish the principal forms. In this manner I have always easily recognized the species of *Pediastrum*, *Closterium*, *Pandorina morum* and many others; indeed, of the first genus, approximately to guess the species.

We often discover a desired species in a test tube of water containing algæ, and wish to subject it to observation. We may best do this by taking a glass tube, evenly cut off at both ends, of about 2–3 mm. interior diameter and closing one end of it with the index finger of the right hand, put the other end slowly down into the water. The inclosed air keeps the water out. Now bring the lower open end of the tube directly over the floating alga, and suddenly remove the finger from the upper end. The water rushes into the tube with great force — all the more forcibly the farther beneath the surface of the water the lower end of the tube is — and carries with it all the objects swimming in the neighborhood and our alga among them. The tube should then be again quickly closed at top and withdrawn from the water. Its contents should be poured

into a watch-glass, and here, in this limited space, the hunt for the alga may be made. Or, if one can see the alga in the tube, by partly opening the upper end, the water may be suffered to run out slowly, drop by drop, till the alga has come down into the underhanging drop, when it may be quickly captured and removed with a slide. Still better, by means of an apparatus, illustrated in Fig. 75, the drop with the alga in it may be brought directly upon the slide. This contrivance consists of

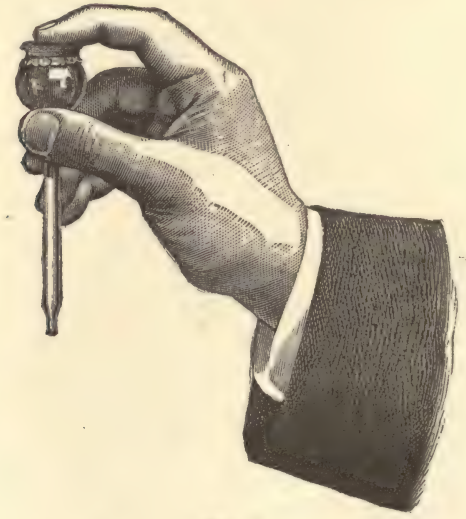


FIG. 75.

a glass tube narrowed at the lower end and widened into a hemispherical form at the top, covered with a small piece of rubber. Its use is understood without further explanation.[5]

B. MACERATING OR SOFTENING.

This method of preparation depends on the fact that plant tissues, which have lain in a certain fluid for a long time, undergo in part a disintegration, while other parts resist the destructive influences and so are parted from each other, and thus separated are fit for examination.

[5] In a long note the author gives particular directions for "blowing" these pipettes, for one's self, out of glass tubing. But it seems scarcely necessary to do that when one can buy them very cheaply of the dealers, or may substitute "dropping tubes" which may be had at any apothecary shop for a few cents. A. B. H.

Very delicate plant tissue may be macerated by lying for a considerable time in distilled water. But the process may be greatly hastened by warming the water. In this way one may obtain the large continuous epidermis of many foliage leaves, particularly of the above named monocotyledons. Finally, other soft parts of plants may be macerated by continual boiling in water. Hartig[6] recommends a well tinned vessel into whose closely fitting cover is soldered a glass tube, a meter long, much inclined from the perpendicular. The steam condenses in the glass tube and runs back into the vessel so the boiling may go on as long as it is necessary without particular care.

A process for macerating the woody organs of plants has been given by M. Schultze, and consists of treating the tissue to be macerated with potassium chlorate and nitric acid. The tissue is boiled some seconds in a flask with nitric acid to which is added a little potassium chlorate. Then pour the whole out into a considerable quantity of pure water and fish out the plant-tissue with a little glass slip, and it can then easily be dissected with a couple of needles (Hartig).[7] Hartig[8] puts small pieces of the cellular tissue to be treated in a test tube with a like volume of potassium chlorate, then pours over it concentrated nitric acid, and warms over the spirit flame to boiling, till the cells separate and then washes out with water. It is scarcely necessary to mention that by this process all cell substances with the exception of some cell walls are destroyed.[9]

According to Hartig[10], artificial freezing mixtures furnish a good means of separating into their elements the cellular tissue of ripening or germinating seeds. A good freezing mixture is produced by pulverized glauber salts and hydrochloric acid by which a refrigeration of —12° without special pains and in high summer temperature is easily obtained. The object should be put in a thin-walled test tube and left in the cold mixture as long as the temperature is below zero.

[6] Hartig, Entwicklungsgeschichte des Pflanzenkeimes, Leipzig, 1858, p. 153.
[7] Hartig, Das Mikroskop, p. 394.
[8] Hartig, *l. c.*, p. 153.
[9] Kabsch in Pringsheim's Jahrb., Bd. III, p. 357, *ff.*
[10] Hartig, *l. c.*, p. 153.

C. Incinerating and Calcining.

It is well known that in the superficial layer of many plants there is intercalated a silicate, by which a considerable rigidity is imparted to it. Since the silicate is not destroyed by burning or by mineral acids it may be easily obtained in the form of a siliceous skeleton, after the more delicate parts of the tissue have been destroyed. For this purpose treat the given tissue (for example, a piece of the epidermis of *Equisetum hiemale*), first, with Schultze's macerating mixture till it is colorless, wash it well and then burn out the residuum on platinum foil.[11] The siliceous skeletons of the Diatomaceæ may be prepared in a pure state by this process. Sachs[12] employs the following method for incineration. "In order to obtain beautiful skeletons it is necessary to soak the detached epidermis, or thin section beforehand, in nitric or muriatic acid, and then burn it on the platinum foil. I have found another method still, much more convenient. I lay larger pieces of the tissue (for example, grass blades, equisetum stems, etc.) on the platinum foil in a large drop of concentrated sulphuric acid and heat it over the flame. The mass at once turns black and a powerful development of gas takes place. The heating should continue till a pure white ash alone remains. This very soon happens, while the common method of incineration almost always takes a good deal of time and does not always produce a perfectly colorless skeleton."

Many cells contain calcareous matter inlaid or onlaid, making them opaque and unfit for microscopical investigation. In many cases the mineral matter (if it be, as is common, calcium carbonate) may be removed by treatment with dilute muriatic acid (species of *Chara*, *Corallines*, etc.). For this purpose put the section or the whole of the plant, as the case may be, for a longer or shorter time in cold acid which dissolves the incrusting calcium with a development of carbonic acid.

[11] Hugo v. Mohl in Botan. Zeitung, 1861, p. 208.
[12] Sachs' Lehrbuch der Botanik, III, Auflage, p. 38.

III. INSTRUMENTS FOR THE PREPARATION OF MICROSCOPIC THIN SECTIONS.

With the exception of the infrequent cases already mentioned the microscopist will be constantly confronted with the task of preparing a very delicate, perfectly transparent section of the organ under investigation. For this purpose different instruments are necessary, and the success of the preparation materially depends upon the good order and proper management of these instruments.

It is, therefore, first necessary to make ourselves acquainted with the tools we are to use before we undertake the preparation of the section itself.

It was formerly the opinion, as v. Mohl has expressed it in his own stiking way,[13] that an artificially augmented power of vision required artificially enhanced capability of hands — that the preparation of the object would become easy by help of a multitude of instruments and artificial apparatus. "With all these, few will be helped. One may train his hand to surer and steadier motions than the simplest apparatus can accomplish. To no one does the saying of Franklin so fittingly apply as to the microscopist. 'A naturalist must be able to saw with an auger, and bore with a saw.'" We must apply these words of the great anatomist in the strictest sense, because we believe that those who are the most skilful preparators, and who attain the best results are those who understand how to work with the fewest and simplest instruments possible. Just as little as it is possible to construct a machine for the calculator — really for many very desirable — which shall lead him mechanically, with mathematical certainty to right conclusions, even so little is it possible to invent an apparatus which in the hands of the unskilful and inattentive will turn out objects for the microscope suitable for investigation.

The instruments commonly employed by the microscopical

[13] H. v. Mohl, Mikrographie, p. 255, *f.*

preparator are the razor, scalpel, lancet, scissors, needles, and forceps.*

A. The Razor. Among the preparing instruments of the microscopist the razor occupies the most prominent place. We employ the form of handle prepared for the barber's uses, with a blade which is movable in the handle.[14] The requirements which a razor should satisfy in order to be really serviceable are somewhat the following. The razor should be strongly made, but at the same time relatively as light as possible. A knife which has a heavy handle or a very massive blade soon tires the hand while cutting. Further, the heel at the base of the blade should be round and blunt edged, not angular and sharp cornered. This — though it is not commonly thought of — is important, because, in cutting, one has to lay the end of his thumb on the heel, and if this be sharp or angular it will after a while scarcely fail to do injury to the skin of the thumb. Finally, the blade should be of the best hardened steel and as broad as possible.

As to the form of the blade, one should choose two kinds adapted to his needs, viz., first, a pretty thick blade which gives, in section, somewhat the form shown in Fig. 76, I. It is ground but slightly hollowing and should not spring when pressed upon the finger nail. For the second, take one ground very concave, consequently of a leaf-like form (section seen Fig. 76, II), which springs when one presses it upon the thumb nail and rings when struck with the finger. The last form is not commonly furnished by the instrument makers, but can be ground out from the first named form. Finally, there is a form recently introduced in which the blade is concave on the upper and plane on the lower side, Fig. 76, III. It is capable of doing

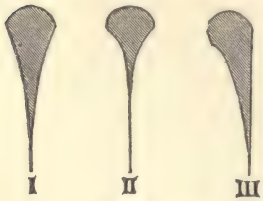


FIG. 76.

* Also some other tools to be described hereafter, including the section instrument or microtome, and of course the straight edged knife. The author does not include the section cutter but it will be found extremely useful in a great variety of cases, and in many quite indispensable. The instrument and its use will be found described farther on. A.B.H.

[14] Razors are furnished by some microscopical institutes which when opened the blade may be made fast in the handle. For cutting with a microtome this might be practicable, but it is not so serviceable in conducting free-hand cuttings.

good service in cutting wood sections, but has the fault of being very difficult to sharpen.

[The J. R. Torrey Razor Co., of Worcester, Mass., regularly manufactures razors exactly answering to the description of forms I and II. Their No. 583 has a blade of the form indicated in Fig. 76, I, and their No. 147 has a very fine, thin, broad blade with a sectional view exactly corresponding to Fig. 76, II. For breadth, finish and quality of blade these razors leave nothing to be desired, and probably excel those of foreign make. This firm also makes, especially for naturalists' use, the plano-concave razor represented in Fig. 76, III. The general form of this razor is shown in Fig. 77. The side not shown in the illustration is the one that has been flattened. It is of medium size, considerably smaller than the others, and is an instrument

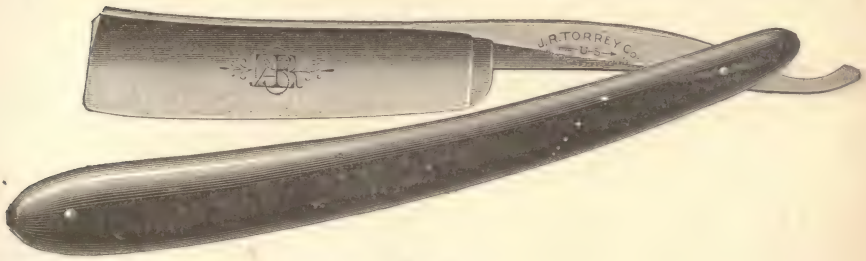


FIG. 77.

so well adapted to the use of the phytotomist that I think he will employ it more than either of the others, not only for making wood sections but also for cutting nearly all those kinds of soft tissue that may be held in an elder pith, and for this reason. I find it a great advantage to hold the elder pith, when once the section material is rightly embedded in it, in a hand-vise. Then by so holding the vise in the left hand that the index finger comes to a position partly surrounding the pith and at a level with the top of it, this flat side of the razor may be laid on the finger in such a way as to make of it a very excellent rest and guide in the cutting, giving great precision to the movements of the knife and enabling the operator to cut extremely thin and very even sections. A. B. H.]

The razor is for the vegetable anatomist what the chisel is for the sculptor or the brush for the painter. He needs it for making almost every preparation, and he must therefore devote the greatest attention to keeping it in order.

If the knife, by some carelessness, has got nicked, the simplest means of remedying the fault is to take it to the instrument grinder. But the right kind of a microscopist will not be dependent on others for every little thing, all the more since by a little pains he can remove the nick himself. For this purpose the knife should be applied to the oil stone till every part of the fault has disappeared. Five or ten minutes will commonly be required for this.

The oil stone is a whetstone of smoothly polished amorphous quartz, the best and hardest being brought from North America.* A drop of olive oil is put on the clean stone, the razor opened and laid flat upon the stone, back and edge resting upon it, and then drawn diagonally over the stone the back forwards. On coming to the upper end of the stone turn the knife over on its back, and draw it, back forwards, again to the place of starting. This should be repeated as long as it is necessary. If the grinding is being rightly accomplished there will be a peculiar sound; the knife must be "drawn" over the stone as the experts say. When by continuous work on the oil stone, the nicks are all removed, the oil stone is to be replaced by a softer one of slate stone, whereon the work is to be continued in the same manner only with the use of water instead of oil.[15] We now have an edge without a nick, but not one quite smooth. One may convince himself of this by examining it with a magnifying glass, when he will see that all along the edge diagonally to the long axis of the blade are fine furrows. These can be removed by the use of the strop.

Of razor strops there are, as is well known, various forms and sizes. There are also very bad and very good ones; only the

* The Arkansas oil stone. A. B. H.

[15] The name of these whetstones is not known to me. [They are sold as "barbers' hones" in this country. A. B. H.] They are about 17.7 cm. long and 4 cm. broad, and consist of a layer of oily-feeling yellowish stone which may be scratched with the knife and which is made fast to a piece of blue slate by some cementing medium. They are to be had in the larger hardware shops for about 1 Mark apiece. If in sharpening the knives the stone gets scratched, it may be made perfectly smooth again by the use of a very fine emery paper.

latter are suitable for our purposes. If one has an opportunity to get one which has been used for a long time by a barber and has by use acquired a perfectly smooth and glistening surface, let him take it; it is far better than any that can be had of the instrument maker in the most elegant case. But one is commonly referred to the article which may be had in the market.

[The firm of J. R. Torrey and Co., of Worcester, Mass., manufactures a razor strop which seems to me to meet all the requirements of the phytotomist. It is No. 700 of their catalogue "The combination hone and cushion belt strop," and is represented in Fig. 78. It has four stropping surfaces, sides 1 and 2 sliding within 3 and 4, the latter two being those most commonly used in keeping the razor sharp. They are rather stiff cushions, No. 3 having a fine black surface for sharpening the edge, and No. 4 a buff, velvety, surface for giving the final smoothing and polishing to it. Side No. 1 is a fine,

FIG. 78.

Italian rock hone which may be used with either water or oil, preferably with oil, as it is best not to run the risk of dampening the wood and leather parts of the strop by the use of water. It will furnish all the sharpening stone the phytotomist will need for his razors. Side No. 2 is a reddish-brown, flat, hard, leather surface, apparently provided with oxide of iron or some such like polishing paste, and is used like any strop, first after honing the blade.]

[The honing is done as already described, though some recommend moving the blade across the stone diagonally from point to heel with the edge *forward* instead of *drawing* it as just mentioned. The stropping of the razor for our work is done precisely as for ordinary uses, laying it flat, edge and back touching the strop, upon the brown, black or buff surface as the case may be, and drawing it back forwards diagonally from heel to point a dozen times or more, up and down, turning it upon

the back each time. In finishing upon the buff surface it is recommended to let it lie not very heavily upon the leather, so that its soft, velvety surface may not tend to round the edge. Before beginning the cutting, the author recommends drawing the edges carefully through a bit of elder pith to remove all adhering particles of grit, or steel, which may have been left upon it from the sharpening process. I find that carefully drawing it lengthwise between the ball of the thumb and first finger of the left hand, when pressed somewhat closely together, will effect the same result. A. B. H.]

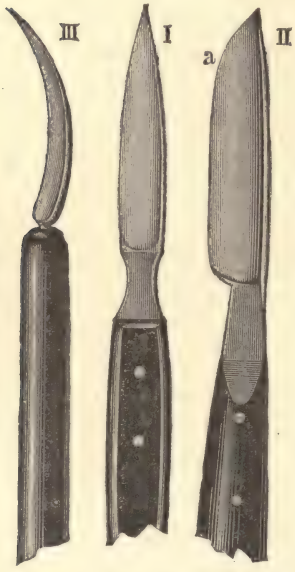


FIG. 79.

For very delicate cuttings, I subject my knife, before the removal of the particles, to a still further process, in order so to polish the edge as to remove the least possible trace of every kind of furrow which the different grindings and stroppings may have left upon it. This polishing, which is commonly a failure with beginners, is done in the following manner. Commercial chalk is sifted through a piece of linen, and on a thick, very smooth plate of glass a little of the sifted dust is mixed with water. Then lay the blade flat, back and edge upon the glass and the paste, and polish with a circular motion. When the first side is finished take the other. If I mistake not, this process of polishing originated with Hugo v. Mohl.

Now, that the razor is perfectly sharp the utmost care should be taken not to let it get dull again. It should never be laid open upon the table except as the blade forms an angle of about 270° with the handle. In this position it is impossible for the edge of the razor to come in contact with the surface of the table on which it lies, so as to be damaged. During the process of section-cutting the razor strop should never be laid aside, but frequently used by giving the knife several

strokes on it to maintain a uniform sharpness. When the work is finished for the time being, all fluids which have been used to assist the cutting should be removed from the knife, using when needful, first water, then alcohol and ether.

B. Scalpels. Of scalpel-shaped knives the vegetable anatomist may employ, with advantage, the sorts illustrated, natural size, in Fig. 79. These knives are not to be employed for making the section, but for properly preparing the parts of the plant upon which the razor is to be used.

The first and second forms are knives with straight blades, differing only in respect to their points. In the case of I, the point is in the middle. This knife is used to prepare pieces of stems, leaves, roots and other parts of the plant held in the hand, before subjecting them to the razor. The knife II has the point lying laterally and forms an angle with the edge. It should be very sharp from here to the point, and is used in trimming down the section as it lies on the slide under the mounting microscope. Form number III, a small knife with slender, bent blade is convenient to use when some peculiar form of organ is to be prepared, parts of which are difficult to get at. It renders its best service in separating very small flower buds when one is studying the history of the development of flowers.

The handles of these knives are of ebony, flat and long, so long that in use they may lie between the thumb and forefinger, say 9, or better 11, cm. long.

The scalpels are sharpened in the same way as the razor. If quite dull they should be first worked on the oil stone and then on the clay stone with water. They should not be turned over right and left, at every stroke as with the razor, but sharpen one side and then the other. The final polish is given on the strop. It is also recommended to keep a small cap of elder pith on the point of the knives when not in use to protect them from injury.

C. Needles and Lancets. Needles of various sizes and strength are among the most important requisites of the microscopist, always, however, being so strong that they will not spring, fastened immovable in a wooden handle, Fig. 80, I, or

set in a brass holder whose cap screws on like that of a lead pencil, Fig. 80, III. They are useful in lifting the section from the razor, in spreading it out when it is folded up on the slide, under the preparing microscope, in tearing apart macerated plant tissue, in removing air bubbles from the mounting fluid, and in many other ways. For these uses the needle points should be slender and sharp.

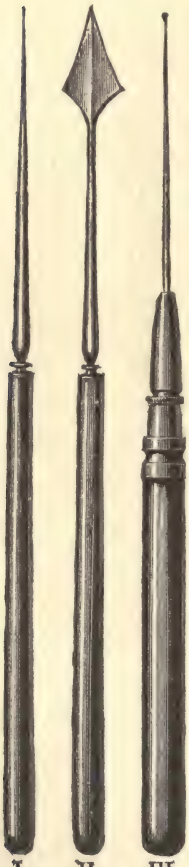


FIG. 80.

Lancets are in the hands of the phytotomist of far less use than in those of the animal histologist. A form of lancet, which can render important service in vegetable histology, is illustrated in Fig. 80, II, the lancet-needle. It is seldom of use for cutting, but in lifting out large sections from considerable quantities of fluid it is almost indispensable. For this purpose indeed, the forceps may be used, or a hair pencil, or a common needle, but by the use of the first the section may be easily injured and by the last it is apt to be folded up. But by the lancet-needle, of the illustration, it may be removed from the fluid in the vessel without injury, by putting the lozenge-shaped surface under the floating section and then suddenly lifting it upwards. Then the section spreads out and may be lifted up since it has more adhesion to the surface of the lancet than for the fluid. The use of the lancet-needle is especially commended when dealing with sections which have been treated with reagents that render them very fragile and easily torn apart.

The needles are sharpened on the stone by turning them rapidly with the handle, as they are being moved back and forth over the stone, dampened with oil or water.

D. Forceps and Scissors. The forceps, represented in Fig. 81, are useful in many ways. They are best made of steel with long and slender legs which are semicircular in section either

straight or a little bent. Their inner surfaces are cut like a file. Forceps of brass or German silver are useful but are to be less commended than those of steel. They have but one advantage over those of steel, that their dulled points may be put in order again with a file.

The scissors are a very subordinate instrument in the microscopical preparation of vegetable objects. Small scissors with slender straight or even bent blades may be found useful now and then.

FIG. 81.

E. Other Requisites. Of the other instruments which the microscopist frequently finds useful in making preparations, we will name the following.

a. Hair pencils. Small hair or India ink pencils, mounted in quills and provided with long wood handles, are useful in lifting the sections from the razor blade, in removing superfluous fluid from the slide, and if perfectly dry, for brushing away dust from slides or cover glasses.

b. Glass rods. These are prepared from longer glass rods or tubes about 4 mm. thick, by cutting off any desired length, which may be done by softening them in the flame at a given point and then pulling quickly apart. The ends should be carefully rounded by melting. They may be 14 to 20 cm. long and are chiefly used for carrying drops of the reagent or mounting fluid to the slide.

c. Porcelain dishes. Small, of 60 to 70 mm. diameter and about 13 mm. deep, with flat bottoms to stand secure, and used to receive the sections, as they are cut, in a considerable quantity of water or other fluid in order to drive out the air by boiling, or in order to treat them with reagents by heat. One should be provided with about half a dozen of these dishes.*

* Common white individual butter plates, which may be had cheap in any crockery shop, answer excellently for these purposes. Choose comparatively thin and capacious ones, of two different sizes, say two dozen each, then one size may be used to cover over the other to exclude dust and retard evaporation from the preparation. A. B. H.

d. *Small porcelain crucible* with cover of 50 mm. diameter and 35 mm. high for the maceration of vegetable tissue (see p. 163).

e. *A set of watch-glasses*, of different sizes, for example of a diameter of 32, 40, 48, 64 and 80 mm. They may be applied to the same uses as the porcelain dishes, and also to finding algæ. A very practical contrivance for keeping sections in many fluids protected from dust and evaporation is represented in the apparatus pictured in Fig. 82. It consists of two watch-glasses of like size with ground edges and held together with a brass clamp, as shown in the illustration. The under watch-glass contains the fluid with the sections.

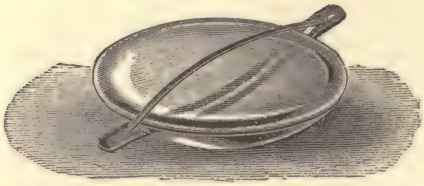


FIG. 82.

[The "Syracuse solid watch-glass," shown in Fig. 82½, is an excellent device, recently contrived by Dr. A. Clifford Mercer of Syracuse, N. Y., for use in the histological laboratory of the Syracuse University. It is made by, and can be obtained from, the Syracuse solid watch-glass company. It is a short cylinder of glass, about 55 mm. wide and 15 high, deeply concave at the top so as to have the internal form and capacity of a large watch-glass, and slightly concave at the bottom in order to stand firmly upon the table or microscope stage. Being transparent and colorless, it is well adapted to be used upon the microscope with transmitted light, or to be placed over white or black paper on the table, according to the background required. As a microscopical bath, staining or dissecting dish, it possesses the well-known advantages of the watch-glass, except where heat is to be applied, with the added luxury of standing solidly on the table, of immunity from breakage, and of fitness

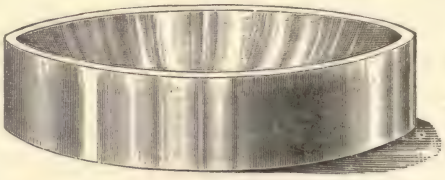


FIG. 82½.

to be piled upon each other for economy of space and as a covering to each other for protection of their contents from evaporation or from dust. The glasses having only the upper and lower edges cut may be used for many purposes, but those having the concave surfaces also cut and polished are preferred upon the microscope stage, especially for the immersion use described on page 32. R. H. W.]

f. *A small wash bottle*, of the form used in chemical laboratories and filled with distilled water, is of great use.

g. *Spirit lamp and tripod*, or in place of the first a Bunsen burner. The tripod should be provided with a brass netting of narrow meshes for supporting the watch-glasses and porcelain dishes in heating the sections, and for maceration with the aid of heat.

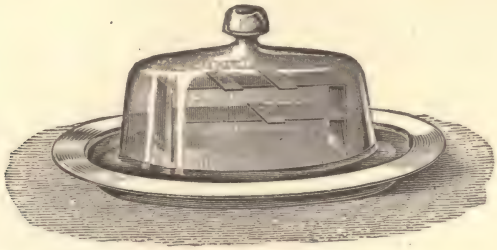


FIG. 83.

h. *Bell glasses.* They are useful in protecting the preparations from dust. For this purpose one should have several of them from 10 to 30 cm. in diameter. They should be provided with a knob on top for convenient handling. They are used simply by putting them over the preparation as it lies on the table or on paper. If it be desired to keep the specimen moist it may be put on a shelf made of zinc, as in Fig. 83, which stands on a plate filled with water. Over this the proper bell glass is put with its edge immersed in the water.

i. *A small calcium chlorate dryer* (of the common laboratory form) which can be conveniently employed in quickly extracting the water from objects which are to be mounted in Canada balsam.

k. [*The spiral spring clip*, scarcely more cumbersome and costly than the common wire spring clips used for holding the

cover glass in position while mounting objects, is the "Nassau" spring clip devised by Prof. Libbey of Princeton College and named after Nassau hall of that institution. In this device, which is made by T. H. McAllister of New York, and shown in Fig. 84, a straight wire presses vertically upon the center of the cover-glass. The wire slides easily through two horizontal folds of metal above, and is held down by a spiral spring that encircles it and presses against a nut screwed upon the wire itself. By twirling the upper end of the wire between the thumb and finger, any degree of pressure may be secured, from barely touching the cover-glass to a force incompatible with its safety. After placing it upon an object, the pressure may often be changed to advantage, trial having shown more or less force to be required. If considerable pressure be needed the bottom end of the wire may be capped with a little block of cork to prevent danger of cracking the cover-glass; but for the lighter pressures this precaution is not requisite.]

FIG. 84.

[*The Parallel Compressor.* For examining thin objects spread out to considerable size, or minute ones diffused sparsely through some liquid, it is nearly indispensable to have some means of lowering the cover-glass upon them gradually, with or without pressure, and without loss of parallelism between the cover and the slide. Some of the parallel compressors made for this purpose have the cover-glass fixed at a certain height, and the lower glass adjustable toward it by a screw and spring motion. With such arrangement, the cover-glass having no upward movement is less likely to be broken by carelessness when high powers of short focus are in use. Such powers, however, cannot be advantageously used with carelessness, in any way.

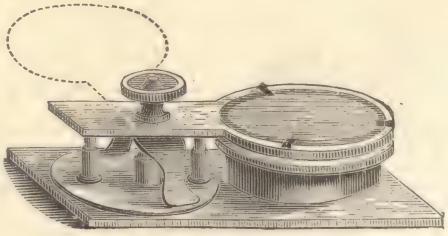


FIG. 85.

For those observers who find a downward motion of the cover, by screwing downward, to be more natural and manageable than an upward movement of the object toward it, the Bausch and Lomb parallel compressor, Fig. 85, offers a very efficient instrument. It has a working aperture of 25 mm., the extreme size of the cover-glass being 32 mm. The cover is carried down by the direct action of a milled nut turning upon a screw, is kept parallel to the plane of the stage by two vertical pins sliding in deep sockets, and can be turned aside, away from the lower plate, by means of a pivot-motion of the arm that carries it. Being attached to the arm by little steel spring buttons, it can be replaced without delay if broken. R.H.W.]

The foregoing list of instruments includes about all those which the operating table of the preparator should never lack. Some other apparatus, employed only on special occasions, as well as the contrivances for the preparation of permanent mounts, will be described in their proper place.

IV. CUTTING MICROSCOPICAL SECTIONS.

Most vegetable organs are best adapted for cutting when fresh. Specimens should be immersed in water as soon as they are collected and likewise after having parts cut from them for preparations. The sections should be made with the cutting surface covered with water and the razor blade also moistened with it, then the section may be prepared by either of the methods described below. Not only are most objects when fresh most suitable for cutting but they are also then in a state most favorable for the study of the cell tissue, or the structure of the cell wall, be it of portions of stalk, leaf or flower.

In another group of forms there are difficulties in the way of cutting the fresh material. Many organs are too soft, delicate and elastic, to offer sufficient resistance to the knife for section making. Others consist of substances, partly hard and partly soft, so that though the former may be cut well enough, the latter will be torn. In still other cases the cell contents will be displaced from their natural position by cutting, In such cases the substance must be previously hardened.

Materials for cutting may be hardened by the use of the following media[16].

1. *Alcohol* in its anhydrous state is more suitable than other fluids, not only for fixing the cell contents, but also for hardening the cell walls. Hardening by means of alcohol is indeed an old process. In recent times it has been employed chiefly by Strasburger to whom it has yielded the most beautiful results in the study of the nucleus and self-division of the cell. The portion of the plant to be hardened should be put into absolute alcohol in which the cell wall very soon becomes rigid, and the protoplasm with slight contraction is "fixed." The more rapidly the fixing takes place, the more natural the relation of the part will remain. Many kinds of delicate tissue will not bear the use of absolute alcohol on account of the shrinking of the membrane caused by the sudden withdrawal of the water by the alcohol. It must, therefore, be applied very gradually. The plant must first be put, for a long time, in a very weak solution, then in a stronger and, finally, in absolute alcohol.

Again, in cutting other objects, we find they behave most satisfactorily when they have lain for a long time in a mixture of like parts of alcohol and glycerine.

When the object is being cut, which has been hardened in either of these ways the razor should be moistened with the fluids used for hardening and the cutting surface kept wet with them.

2. *Perosmic acid.*[17] A one per cent solution of this acid "sets" the protoplasm by instantaneous hardening still more quickly than absolute alcohol. In more recent times it has been employed by many naturalists for this purpose. Many preparations which become opaque by the use of alcohol retain their full original clearness by the addition of one per cent perosmic acid.

3. *Chromic acid solution* and *Potassium bichromate.*[18] The former should be a one per cent solution, the latter in different degrees of dilution, in water, and it is suitable for the

[16] Nägeli und Schwendener, Das Mikroskop, p. 476.— Sachs in Bot. Zeitg. 1864, No. 11, 12. —Dippel, Das Mikroskop, Bd. I, p. 282.—De Bary, Vergl. Anatom., p. 86.—Strasburger, Befruchtung u. Zelltheilung, 1878, p. 38.— Strasburger, Zellbildung u. Zelltheilung, 1880. p. 9, u. a.—Poulsen, Bot. Mikrokemi, p. 19, *f*., deutsche Uebersetzung, p. 23 *f*.

[17] Frey, Das Mikroskop, p. 103, *f*.—Strasburger, Zellbild. u. Zelltheil. 1880, p. 39, 172, u. a.—Poulsen Botanisck Mikrokemi, p. 15, *f*., dtsch. Uebers. p 18 *f*.

[18] Hanstein in Bot. Zeitg., 1868, p. 697, *ff*. — Dalmer, Ueber die Leitung der Pollenschläuche, p. 18 (Jenaische Zeitschr., Bd. XIV, N. F. VII, 1880).—Poulsen, *l. c.*, p. 14, 31, dtsch. Übersetz., p. 17, 37.

hardening of many preparations which contain gums and other carbohydrates, resin, etc. Resin for the most part would be dissolved in alcohol, while many kinds of gum by hardening in alcohol are thrown down as a white opaque precipitate. In the preparation of the potassium bichromate solution one may use the commercial salt having purified it by recrystallization.

We now proceed to describe the different kinds of microscopic sections as well as the methods of their preparation.

FIG. 86.

1. FREE-HAND CUTTING.

The razor should always be held, when cutting, in the right hand, in the manner shown in Figs. 86 and 87, the blade forming an angle of 112° to 130° with the handle. The hand grasps both handle and blade, all five fingers being engaged in holding the instrument. The thumb lies with its point in the throat of

the blade, the end of the handle lying in its first joint. The index finger grasps the end of the blade from above with the end thrown around beneath. The handle rests in the metacarpal part of the hand and is clasped by the first and middle phalanges of the middle, gold, and little fingers. The center of gravity of the knife lies now within the clasping hand. All the fingers firmly grasp the knife.

Transverse Sections. We will now suppose we are to prepare a section of the stem of some plant. We take the object, which has previously been given a good cutting surface by means of the scalpel, with the thumb and forefinger of the left hand as shown in Fig. 87, so that the cut surface is but a trifle above

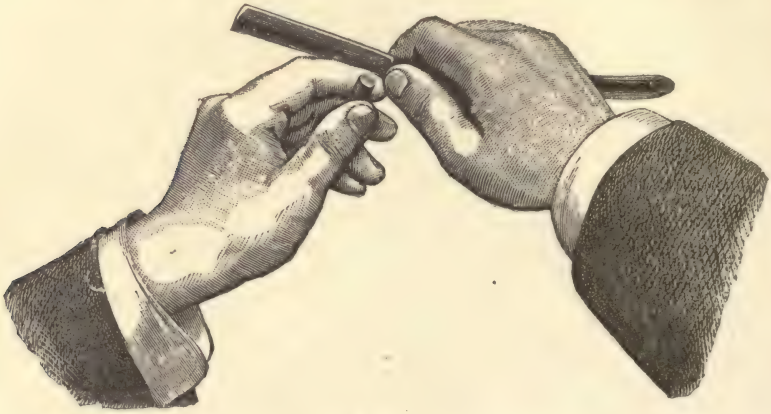


FIG. 87.

the end of the thumb. Now lay the knife flat on the index finger of the left hand, its edge near the heel perpendicular to the long axis of the stem. The cut may now begin. If now we push the knife directly through the plant like a wedge, we shall find that we have a perfectly worthless section. It will be more bruised than cut. We must cut with a diagonal motion, drawing the knife in the direction of its longer axis, from heel to point, at the same time that it is pressed forward through the stem.* In this way all parts of the edge come in contact with

* I have to confess that in my experience I can do better work by moving the knife in the other direction, viz., from point to heel. I suppose the reason is because, in sharpening the knife, it is drawn from heel to point, so that if there are any microscopic lines along the edge, or teeth-like projections upon it, they are placed in such a position as to take hold better when the knife moves through the substance which is being cut, diagonally, from point to heel. A. B. H.

the section, which will be found finally at the upper end of the knife lying on the moist blade.

It should now be lifted from the knife. This may be done by different instruments, the forceps, the hair pencil, the needle or the lancet-needle. Only thick sections can be handled with the forceps, such as are not injured by the unavoidable pressure involved in seizing them with the forceps, or such as we care to examine only certain parts of and so do not mind the other parts being more or less injured with the forceps. The hair pencil is on many accounts to be recommended for handling sections. In some cases it is very suitable and in others not, since in sections with large cells the capillary attraction which attends its use is quite likely to draw out the cell contents. The simple needle avoids this, but in lifting up sections with that, large sections, at least, roll themselves up about the needle and so entangle themselves that it is very difficult to put them in order again. This difficulty will be avoided by the use of the lancet-needle, Fig. 80, II. When the section lies on the blade of the razor and a *large* drop of water is added it will soon begin to float. Then by putting the leaf-like expansion of the lancet-needle under it and lifting it quickly upwards, the section will lie flat upon the needle and may be immediately transferred to a dish of water or the slide. [If the section has been cut in, and is to be transferred to water, a still better way is to hold the razor blade inclined over the dish and direct upon the section from the wash bottle a small stream or a few drops of distilled water which will float it off into the dish; and it often happens too that a section upon the slide, which has got folded or rolled up and is too delicate to bear handling with the needles, may be perfectly spread out by directing upon it for a few seconds a fine stream of water from a wash bottle. A. B. H.]

Longitudinal Sections. In making longitudinal sections a little different method will be employed. According to the nature of the material to be cut the longitudinal section will be made *between the fingers* or over the finger.

The longitudinal section between the fingers is made in the following way. A small moistened piece of the object to be cut lengthwise is taken between the thumb and index finger of

the left hand, Fig. 88, or if it is too bulky then a prepared thin piece of it. Now, taking the razor, not as before, but in the manner shown in Fig. 88, that is, with the moistened blade held exactly perpendicular, the lower end of the edge is placed upon the object held between the fingers in such a way that it forms an angle of about 45° with the long axis of the object. The razor is now drawn slowly through it between the thumb and finger from bottom to top, with the application of a gentle pressure. Then we have on the right or left side of the knife, or clinging to the thumb or forefinger, the two longitudinal halves of the object. If neither of these is still delicate enough for examination the operation must be repeated. Many people can in this way cut out a longitudinal section of a very small spherical object.

FIG. 88.

Only such objects are suited to making a longitudinal section over the finger, as are both long and tough, as, for instance, slender stems, under-ground and aërial roots. Take a piece about 4 to 7 cm. long and making two cuts in it near the middle, about 1 cm. apart, half way through it, and breaking it down at these places lay it across the forefinger of the left hand, the portion between the cuts exactly across the back of the finger. The ends should be bent down and held fast by the thumb and middle finger. Now take the razor as shown in Figs. 86–7, and cut a plane surface on the piece lying between the two cross cuts, after which very delicate sections may be made.

2. Section-cutting with Elder Pith and Cork.

The parts of many plants are much too delicate, others too small to be held between the bare fingers during the cutting. There is indeed a whole series of objects which the trained microscopist can cut between his fingers without injury, which the beginner always crushes and only slowly learns to hold softly in his fingers. But there is still, even with the most experienced, a considerable series of objects which he must hold in elder pith, or more rarely in cork, in order to cut them. Belonging to this group are a few objects with which the writer has had no little experience, cross sections of the style and longitudinal sections of the stigma, nectary, ends of roots, the younger and even very youngest states of whole flower buds. These are most conveniently held in elder pith. One may easily prepare this for himself. [But it is much more convenient, and if one's time is worth anything, much cheaper to buy it at the watchmaker's. A. B. H.] In a large bush of *Sambucus nigra*, he will find a large number of already dead and therefore dry branches. From these he can easily obtain the pith by splitting off the bark and wood of the stem, in pieces 5 to 10 cm. long and 8 to 15 mm. thick. Since the pith cylinder is somewhat harder where it joins the wood than elsewhere, one can, in cases where it is desirable, cut this away with the razor while it is being used.

Inclosing the object in the elder pith is done in the following way. Taking a piece of the pith it is cut off even at one end, then split down, perpendicularly to that surface, from 4 to 10 mm. with the razor. Now if one has a cylindrical object to be cut across he drives a stout steel needle perpendicularly into the pith, in the middle of the slit. Thus he makes a hole which by working the needle about may easily be made large enough to take in the object to be cut. The pith is now dampened by being plunged into water while the object, which is already in that fluid, is taken and pushed into the hole until it sticks fast. Since this hole, taking the form of the needle, grows somewhat narrower towards the bottom, there is no risk of

crushing or injuring the upper part from which the section is to be made. Now cut off the object exactly even with the upper surface of the pith and again moistening both blade and specimen begin the cutting exactly as described above. The section together with the surrounding pith is transferred to a cup or watch-glass of water and afterwards fished out for examination.

If a longitudinal section is to be made the beginning of the operation is the same as before. After that a horizontal notch should be cut in the top of the pith, in the direction of the slit, as near as possible of the size and form of the object. For this purpose the scalpel illustrated in Fig. 79, I, will be found useful. The specimen is then placed in this receptacle and by a gentle pressure held fast. One can by experience learn how to increase the pressure on the pith in holding larger objects, for receiving which also one may cut a wedge-shaped piece, some 10 mm. thick, out of the end of the pith, instead of making the perpendicular slit.

Elder pith is almost always to be preferred to cork, as well on account of its softness as on account of its uniform quality. All kinds of cork, even the best, contain here and there dark, hard concretions which make the razor diverge when it strikes against them in cutting. Besides this the cork has a peculiar, even though but a slight, toughness which presents to the beginner no small difficulty of handling. But cork has some advantages in cutting longitudinal sections of, for instance, the ends of roots, as suggested first by Nägeli, if we mistake not. From a large cork stopple make a disk some 10 mm. thick and on its upper narrow edge, by means of a thick gum arabic solution which dries quickly, glue the end of the root to be cut. Then bend over the part of the root which is not fastened and hold it down with the thumb upon the cork, at the same time grasping the other side with the index finger. It is very easy now to cut delicate longitudinal sections of the part which has been glued upon the cork, in the way already described.

[I am inclined to believe that no microscopist ever uses cork in section-cutting except in the way last described, certainly

not as a substance to be cut through. It might, however, sometimes be used as a backing to hold some things up against and cut upon. I have found elder pith, on the contrary, almost indispensable in the free-hand cutting of every kind of the softer vegetable tissue, stems and leaves as well as the most delicate parts of flowers. As convenient a way as any I have ever tried in using it, to cut transverse sections, is to split the elder pith entirely through lengthwise near the middle, then carefully hollow out a place on the flat side of the larger part, in the middle, parallel with the longer axis, lay the specimen in, and over it the slice of pith which has just been cut away, holding the parts together between the fingers while cutting; or, cut a wedge-shaped longitudinal piece out of the pith, cut off the sharp end of the wedge, hollow out and enlarge the bottom of the V-shaped cleft in the pith as much as is necessary, lay the specimen in and replace the truncated wedge. In order to guard against any danger of injuring the delicate tissue by the moving of the parts of the pith upon each other, a little rubber band may be instantly sprung around it, or a thread wound tightly about it and tied, or, as mentioned on page 167, one may hold the elder pith with the specimen in it, in a hand-vise. Then the cutting may proceed at leisure, and one may lay the specimen down to get the use of his left hand in manipulating the section, already cut, without fear of deranging it. It is clear also that specimens for longitudinal sections, of plant parts not longer than the diameter of the pith cylinder, may be fixed in the same way by cutting out the bed for the specimen transversely across the flat side of the semi-cylinder. The pith can then be cut as before, transversely, at least as far as to the middle of the specimen. A. B. H.]

3. Section-cutting in Embedding Media.

A number of things which are to be cut are so small as to be almost or quite invisible to the naked eye. These should be previously mixed in a considerable quantity in a hardening medium and then by making random cuts through this the small

objects will be cut into fine layers. Botanical objects of this kind are such things as starch grains, pollen grains, spores, and delicate leaves of mosses and liverworts. Making sections of such embedded substances is done in the following way. To a thick solution of gum arabic add some concentrated glycerine.* Put a drop of it in a watch-glass and knead up with it, into a tough dough, a quantity of starch grains. Put this in several layers and in sufficient quantity upon the end of a cork stopper and leave it to harden. When the mass has become sufficiently hardened to be cut with the razor, the cutting should be done. Then put it in water and in a few moments the gum will dissolve away and the dissected grains will float out free.[19]

From several sides lately there have come commendations of glycerine jelly as an embedding medium. According to some experiments of my own in this matter I believe that for many botanical purposes it may really be commended although in order to give a definite judgment, these experiments must be carried further in the future. In investigations into the history of the development of flowers glycerine jelly may hereafter be found a medium of extensive application. It is frequently very desirable in this investigation to keep the parts of the flower, where for instance a section of a very young bud has been made, in their relative position when they are put under the microscope. This is very difficult to do when the cutting has been done with water or alcohol. But this is often accomplished where the object has been impregnated with gelatine previous to cutting. It should be put first of all in the gelatine and then by a process to be explained below, freed from air and the gelatine forced into the spaces between the floral parts. The process, however, is somewhat detailed.

Koch[20] has given a process for embedding small and delicate parts of plants in a mixture of tallow and paraffine in order to

* Probably the well known Farrant's Medium would answer this purpose sufficiently well. It is made as follows. Dissolve 4 parts by weight of picked gum arabic in 4 parts cold distilled water and add 2 parts strong glycerine, strain through linen and keep in glass stoppered bottles with a little camphor gum. A. B. H.

[19] Hartig, Entwicklungsgeschichte des Pflanzenkeimes, p. 87.

[20] Koch, Untersuchungen Ueber die Entwicklung der Cuscuteen, 1874 (Botanische Abhandl. herausgegen, v. Hanstein, Bd. II, Heft 3, p. 24).

cut them into sections, a process which has been in manifold use in zoölogy for a long time.

He says: "The mixture of like parts of tallow and paraffine has so low a melting point and stiffens so rapidly, that the plant laid on it loses scarcely any water and therefore suffers no appreciable shrinking. Before putting the specimen into the mixture, it is well to dip it for a minute or less into alcohol and then let the alcohol evaporate. The purpose of this is to remove any water that may be attached to the outside and allows the melted medium to take fast hold of the specimen. If this is neglected, bubbles and spaces will be formed about the specimen which will make it go badly with the section-cutting. A quantity of the melted medium is put upon a slide and the specimen immersed in it. After some minutes it will become sufficiently solid to proceed with the cutting.

For the removal of the fat from the section, they should be first placed in benzole and then in alcohol, and at last treated as fresh sections with reagents.

An essential requirement in the application of this method is that the embedding medium shall have a consistency proportionate to the nature of the material to be cut. This may be attained by changing the relative proportions of the tallow and the paraffine. While like parts of each make a medium suited to the harder parts of plants, the softer parts require a mixture of one part of paraffine to two of tallow. This proportion may be carried still further.

Like results are reached when very delicate objects are treated if they are cut before the mixture is quite hardened. Finally, if fresh plants are to be cut they should be immersed in alcohol for at least a day. But these cut but poorly at best in this way."

[4. Cutting Sections with a Microtome.]

[Our author has given in the text no account of what American microscopists find to be an almost, and sometimes quite, indispensable aid to their investigations, viz., a good section-instrument, or microtome.]

[*The Common Section Machine.* The more common forms are represented in Fig. 89. The material to be cut is put into the well and either embedded in paraffine or other like substance, or packed close and firm with elder pith, and the cutting is done with a very sharp, straight-edged knife, Fig. 92, moving directly forward or diagonally upon the even surface of the metallic top. The material is raised in the well after each stroke of the knife, so as to cut a section of any desired thickness, by turning the large milled and graduated screw head at the bottom, which moves a piston in the well, upon which the material rests. *A* is provided with a set screw at the side designed to turn in and fix the specimens firmly against the

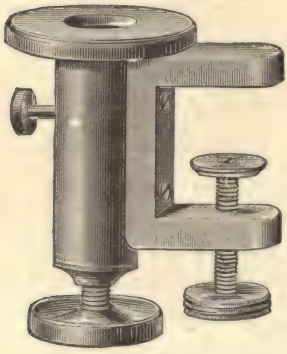


FIG. 89*A*.

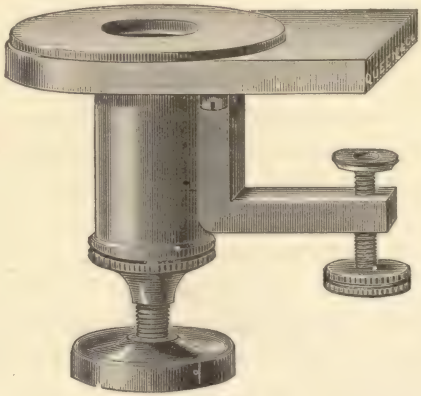


FIG. 89*B*.

wall of the well. This is more particularly designed for cutting wood sections which would need no other means of keeping the material in place. *B* is intended for cutting larger sections of softer embedded tissue. It has a larger well and is provided with a glass top. Both have strong clamps by which to fasten them to the table when being used. A razor instead of the knife might sometimes be used in cutting soft tissue with *B* provided the razor have a very stiff blade, and a very straight edge.]

[*The Providence Microtome.* So called from being the joint invention of several gentlemen of Providence, R. I. The original form was first designed by Mr. N. N. Mason of that city,

who has used it for several years in zoölogical work. The instrument was perfected by Rev. J. D. King, Microscopist of the Martha's Vineyard Summer Institute, one of the gentlemen referred to, by whom it is manufactured and sold.* In its present form it is perhaps equalled by no microtome made, for extreme precision of movement and consequent accuracy of performance in cutting sections. With a good knife in good order, sections of 10 μ to 25 μ thick can be made without difficulty, and all alike. It consists, as is seen in Fig. 90, of a heavy iron bed, *B*, a knife carrier, *A*, and the usual apparatus for holding and moving the object to be cut, *g j*. The iron bed which furnishes the clamp *k*, and a solid support for the knife carrier and object

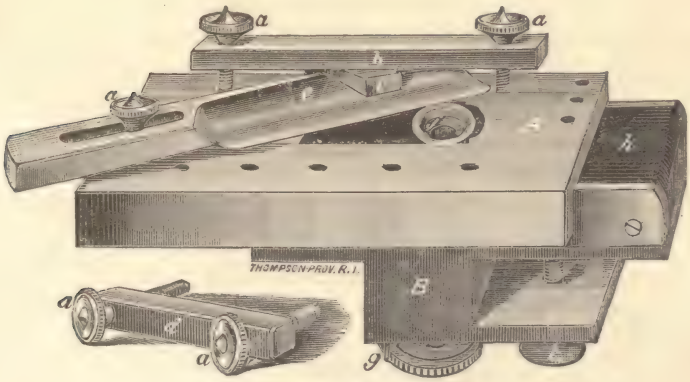


FIG. 90.

holder is 13.8 cm. long, and 5.7 cm. wide, 6.8 cm. deep. Cemented to its top is a brass plate, *h*, 6.5 mm. thick. Rising through and above this is the cylindrical tube or object holder, *j*, 29 mm. in diameter. It projects 10 mm. above the surface of the brass plate and to within 0.5 mm. of the upper surface of the knife carrier. It has an inner cylindrical piston 15 mm. in diameter and a sleeve around this which may be used with the piston, when it is desired to have a larger well, having a diameter of 19 mm. On each side of the brass plate and rising 1 mm. above its upper surface is an iron bar 7 mm. thick run-

* Address Rev. J. D. King, Cottage City, Mass.

ning the whole length of the bed and screwed fast to it. These are the ways or tracks upon which the knife carrier slides. The knife carrier consists of a solid plate of brass 13 cm. long and 8.6 cm. broad, 8 mm. thick, with projections along both sides 6 mm. thick and 13 mm. deep, which fit down over the outside of the iron ways just mentioned. The inside of these projections and the adjoining under surfaces of the brass plate are planed and polished so as exactly to fit over and upon the smooth iron tracks in such a way that the carrier moves freely but with the utmost precision back and forth upon them.]

[The brass plate, *A*, has an oblong opening cut in its middle 9.6 cm. long and 3.3 cm. wide, through which, when in place, the cylindrical object holder projects, very nearly to the upper surface of the plate. The plate is provided along its sides and ends with a series of screw holes, to receive the milled head screws, *a a*, of the clamps, *b d*, by means of which the knife, *e*, is made fast to the carrier, and may be set at any desired obliquity to the line of motion of the carrier. The knife has a heavy strong plano-concave blade with a straight edge, and is laid flat upon the carrier and securely clamped down at heel and point. It, therefore, will not spring in the least and may be depended on to do work of very great precision. It is used for cutting all kinds of wood sections, and such other tissue as can be cut by simply packing in elder pith or embedding in paraffine. The method of embedding in paraffine mixtures for which I am indebted to Rev. Mr. King is as follows:]

[Screw up the plug to within 2 mm. of the top, and fill with vaseline made as hard as can be used with paraffine. Stick the end of the object to be cut in the vaseline, giving the object the desired position. Then screw down till the upper end of the object is below the surface of the plate, and pour in a small quantity of melted paraffine — too much will melt the vaseline and displace the object.—When this is hardened, fill the well with paraffine. The paraffine should be softened with vaseline or, better, paraffine wax. No rule for proportions can be given, as the consistency of the embedding medium must depend upon the hardness of the object to be cut. It should be harder in the summer than in the winter. The temperature too must be de-

termined by the experience and judgment of the operator. If the paraffine is too hot it will boil up in the well and will not cool solid, if too cool it will not properly fill in around the object. If the object is very porous it will be well to give it a coating of mucilage and let it dry before embedding in paraffine as this will keep the paraffine from filling the pores.

When all is complete in the well, the paraffine may shrink. In that case, make a very thin plug of wood and drive it down by the side of the paraffine quite to the bottom of the well. When the section is cut, put it in distilled water, when the section will drop out clean from the paraffine. But if this fails soak in strong alcohol.]

[*The Taylor Freezing Microtome.* As has already been indicated in the author's account of free-hand cutting, there are many vegetable tissues of too soft or too delicate a nature to furnish sufficient resistance to the knife to be cut into sections. He, therefore, suggests certain means for hardening the tissue. But these hardening processes take time and care and are at best not very satisfactory or successful. The zoölogist has long ago met the same difficulty in dealing with animal substances, and has contrived various means of overcoming it. For some time past he has been employing artificial refrigeration as among the best and most expeditious means of hardening tissue. Any tissue, no matter how loose, or soft or delicate in its normal state, if permeated with aqueous fluids, can be frozen solid and cut with a razor or in a microtome like any other solid substance.]

[So far as I know, botanists have made very little use of this extremely simple and effective method of temporarily hardening, for purposes of section-cutting, the soft and delicate tissue with which their studies are often concerned. And yet it is susceptible of a far wider and readier application to the needs and uses of the vegetable, than to those of the animal histologist; for, all fresh vegetable tissues are in nature completely permeated with aqueous fluids. They, therefore, require no previous preparation to make them ready for the freezing process, and parts of dried plants only need soaking in water till they are permeated and softened by it; and vegetable tissue so far as

I can discover is in no way injured, or in the least degree changed by the freezing.]

[The process is almost equally applicable to all kinds of soft vegetable substances, the leaves and stems of the higher plants, parts of buds and flowers, ovaries and ovules in every stage of development, soft portions of roots, tubers and root stalks, the flesh or rind of vegetables, fruits, and soft seeds, pollen grains, spores of cryptogams, fronds of algæ, lichens, hepaticæ, ferns, fungi, etc.]

[Various means have been proposed and adopted for producing artificial refrigeration, as, for example, chemical mixtures; the rapid evaporation of volatile liquids such as ether and rhigoline; mixtures of alcohol and ice,* salt and ice, etc. Several different microtomes have been devised especially for utilizing one or the other of these methods of freezing. By far the simplest and most convenient of them is the Taylor freezing microtome, represented in Fig. 91. It is the invention of Dr. Thomas Taylor, Microscopist of the Department of Agriculture, Washington, D. C.]

[*A* is a revolving plane of glass and brass cemented together, about 10 cm. in diameter, and securely fastened to a short cylinder, 5 cm. in diameter, which screws upon another cylinder which in its turn is made fast to the wooden base. Inside the cylinder upon which the plane revolves and isolated from it is a cylindrical brass chamber 3.9 cm. in diameter, fastened to the wooden base and entered by two metallic tubes, the larger one connected by the rubber tube *t*, with the pail *a*, placed upon a convenient bracket or other support above the level of the microtome. To the other tube is joined the rubber tube, *t b*, which discharges the cold liquid into the pail placed beneath it.]

[The edge of the revolving plane, *A*, is graduated and the movable pointer, *e*, indicates how far the plane has been revolved in a given instance. The screw thread of the cylinder measures about 0.634 mm. So that if the plane is revolved $\frac{1}{10}$ of its circumference it would be raised or lowered as the case might be, 0.0634 mm.; or $\frac{1}{20}$ would elevate or depress it 0.0317 mm.;

* Prof. S. H. Gage in Science Record, Apr. 15, 1884, p. 134.

$\frac{1}{50}$, 0.01268 mm.; $\frac{1}{100}$, 0.00634 mm. Thus by a simple calculation it is easy to determine how far to turn it to get any required perpendicular motion, answering to the desired thickness of the section.]

[The freezing is accomplished by thoroughly mixing coarse salt with snow or finely pulverized ice in the upper pail. Pour on a little water to start the flow of the freezing liquid. When the metallic chamber, which is immediately filled with a liquid at a temperature of about —18° C. becomes frosted over with the moisture condensed from the atmosphere, the instrument is ready for work. The flow of the cold brine may be best regulated by using a faucet or stop-cock with the pail *a*, by which any desired quantity of water may be permitted to pass. The water should be poured back from the lower pail as it runs low in the upper one. This may be repeated as long as it remains below the freezing point. A vessel holding 10 or 12 litres full of snow and salt with perhaps ½ a litre of water poured on at the start will furnish refrigeration sufficient for four or five hours' steady work.]

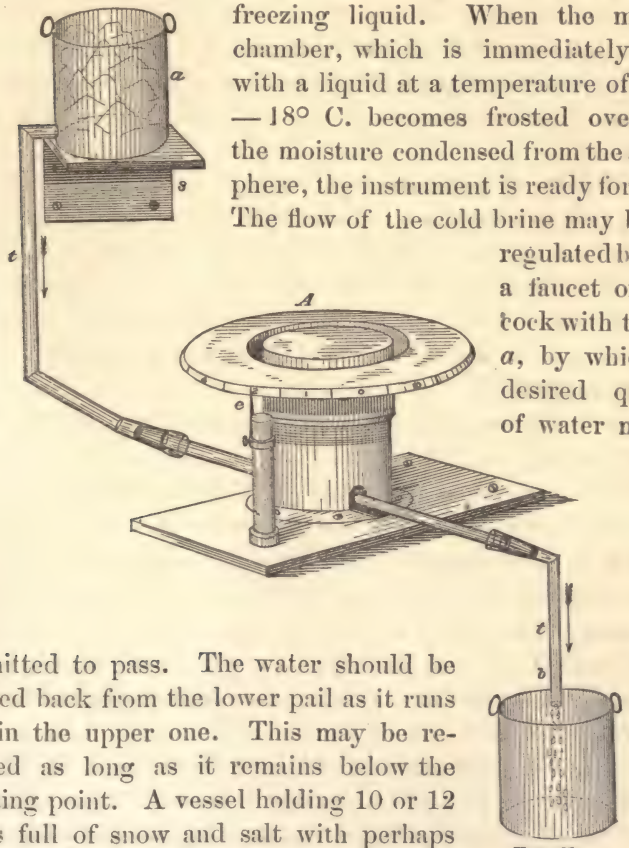


FIG. 91.

[The method of using this microtome is extremely simple. Keep on hand a thick syrupy solution of pure gum arabic,

which after dissolving has been strained through a linen cloth, and to which has been added a little carbolic acid to prevent the growth of fungi. Put a drop or two of this solution on the top of the freezing chamber and immediately immerse in it the tissue to be cut, which has been previously moistened with water, being careful to cover it well with the gum solution. In two or three minutes the whole will be frozen into a grayish, solid, even crystalline-appearing mass, and the cutting may now begin. Now turn the revolving plate backwards till it is brought nearly to a level with the top of the frozen mass upon the cylinder. Then moisten the glass surface liberally with water, and holding the knife upon it as directed, with a straight motion, cut away the top of the mass and make it ready for taking off a section. Now turn the revolving plane forward the right distance to lower it the desired thickness of the section, and with the knife make a diagonal or directly forward, steady, even, but not too quick cut. Remove the section from the knife with a hair pencil dipped in water, into a dish of water provided for it. Or if the section is a small one of tissue not too delicate five, ten, or more may be made and allowed to accumulate upon the knife before being removed.]

[The advantages of this process of hardening and cutting vegetable tissues are so manifest as hardly to require mention. No other method of hardening leaves the tissue and cell contents unchanged, and no free-hand cutting can make the sections of so uniform thickness, or determine with any approach to exactness what the thickness shall be. By this method, which combines in one act the embedding and hardening, the section can be made in any direction across the specimen at will, transverse, tangential, radial or diagonal; and, if the object is not too large, separate portions may be so placed on the freezing chamber as to allow of all these different sections being made at the same time by one stroke of the knife.]

[In order to secure a somewhat greater precision in the adjustment for thickness of section, I have made some changes in my own instrument, changes which, however, in no way affect the nature or value of the extremely simple and effective refriger-

ating apparatus. I make the glass plate fast and the freezing chamber movable. Turning the glass plate down to the limit of the screw it is tightened up and left permanently in that position. By fastening a ring of hard wood inside the outer cylinder, I furnish a support for the freezing chamber which holds it firmly in the center of the cylinder, but permits it to move freely up and down. Beneath all, the wooden base is cut away so as

FIG. 92.

to give the freezing chamber, its support, pipes, etc., space for moving about 15 mm. The chamber and its attachments are made fast at the bottom to a small brass cylinder about 4 cm. high and 18 mm. in diameter, into the center of which from below works a micrometer screw with a thread 1.058 mm. wide. This has a large graduated head, by turning which the freezing chamber with its section material may be raised any desired distance from 5 μ upwards and the section made.*]

[*The Section Knife.* The knife used with these instruments is the common, straight-edged section knife sold by the opticians, and illustrated in Fig. 92. Two things are to be required of it. The one is that the edge be as nearly absolutely straight as it is possible to make it, and the other is that it should be of such quality and temper of steel as that it will take and hold the finest, smoothest and keenest edge possible. In using, it should be held at perhaps an angle of 30° to the horizontal plane of glass over which it moves. A. B. H.]

*Two microtomes recently introduced into this country, the "Thoma Sliding Microtome" from Germany, and the "Caldwell Automatic Microtome" from England, are being used with much favor in some of our zoölogical laboratories. They are chiefly valuable for the extreme tenuity of the sections which they are capable of cutting from the comparatively delicate material of the animal organism, and for the special contrivance in the "Caldwell" form for cutting "ribbons" of consecutive sections. But for work upon the more difficult material of the botanical laboratory I do not consider them as practically useful as either of the two described above, while neither of them has the freezing apparatus, which makes the "Taylor Microtome" so valuable in dealing with the soft, water-holding tissue of many fresh vegetable organisms; besides, the foreign are much more expensive than the American instruments. A. B. H.

V. FURTHER TREATMENT OF THE SECTION.

Having made the sections and immersed them in water, we will now undertake to show how they are to be further treated before they are put under the microscope for examination. [A little instrument shown in Fig. 93 will be found very conven-

FIG. 93.

ient in lifting sections out of the water and indeed in transferring them from any fluid to another, or to the slide. It is a little trowel or lifter, metallic and nickel plated. A. B. H.]

A. REMOVING THE AIR.

We shall observe that in many sections the cellular tissue is partly filled with small air bubbles. Cells filled with air show a thick black outline on the inner wall which prevents the natural structural relations of the wall from being fully made out if at all. It is therefore first of all necessary to remove these minute bubbles from the tissue. It may be done in the following ways, sometimes best in one and sometimes in another.

(*a*) Boil distilled water in a porcelain cup, let it cool and then put the sections into it. The air will soon be absorbed in the deaërated water and after a little time will disappear entirely. This is in most cases an infallible means — only remembering to always use freshly boiled water.

(*b*) The air will be drawn out more forcibly by putting the sections in distilled water and then bringing the water to the boiling temperature, and afterwards allowing it to cool. Delicate specimens, and those whose cell contents are to be studied will not bear the heat.

(*c*) Sometimes the air disappears when the section is removed from the water into absolute alcohol for a considerable

time and then returned to the distilled water. This method was first suggested by Schacht.

(*d*) The air may be removed by means of an air pump. For this purpose many, and, for the most part, very expensive contrivances have been proposed. But, as according to our opinion, he is the best experimenter who does his work with the simplest possible apparatus, we will describe here a very simple but serviceable contrivance, which one can easily make for himself. Fig. 94 represents a thick walled glass tube *a a* of about 22 mm. interior diameter and 15 to 18 cm. long, with one end closed. A small piston of strong zinc plate is fitted to this by winding with tow or other packing, and has a small hole in the middle covered with an air-tight clapper valve *v*. The piston has a handle *h*. Into the cylinder is put a little boiled water with the air-containing section *c*. The piston is now driven down to the level of the water and again drawn up. The valve closes, the space beneath becomes almost a perfect vacuum, and into this the air from the section streams up in the form of minute bubbles. The contents of the tube may now be poured out into a cup and the section removed to the slide.

FIG. 94.

B. Handling the Section under the Preparing Microscope.

The section having been carefully freed from adhering air, it is placed in a drop of fluid, water, glycerine, etc., according to circumstances, on a slide. It will often be found impossible to prevent the edges from being folded under or over. The preparing microscope is used to properly spread it out for examination, which is not by any means a difficult thing to do. Clamp the slide to the stage in such a position that the object will come over the middle of the opening in it and then adjust the mirror so as to throw up a beam of light. Adjust the lens and examine it to see if it is all right, and if not how it is wrong. By focussing up and down it will be easy to tell what parts are

folded over and what under, if any. Then take a needle in each hand and with that in the left hand hold the section down gently and with the other arrange the folded parts. If only a part of the section is to be examined and the others are folded over it, remove them entirely from the slide. This may be done by means of the lancet-needle, or the small scalpel, or even the scissors, holding down the section with the needle as before. Having now rightly laid out the section to our satisfaction, we should put a cover-glass over it. The section can now be transferred to the compound microscope to be studied. If this cannot be done at once it may be kept as long as desirable in an apparatus illustrated in Fig. 82.

C. The Clarification of the Preparation.

Many sections are quite too opaque for certain investigations be they prepared ever so carefully and with never so much skill. They must therefore be subjected to treatment which shall give them the desired transparency. We call this clarifying the specimen. The process is usually applied to those sections which have many untransparent elements, and consists of dissolving out these substances by means of reagents. Since most of these clarifying substances are strong alkalies or acids, it follows that, relatively, but a few classes of objects can bear the process, and of these only those which shall be examined as to the coarser histological relations of their tissue. We should say that this method of clarifying is inapplicable to all those objects which are to be investigated as to some soft cell-contents or some fine cell-wall structure.

There are some substances which produce clarification very gradually. Glycerine is known to be such a substance, and I may add also, according to my experience, carbolic acid and creosote. Sections which have lain for a long time in glycerine become gradually more and more transparent. Sections of stigma tissue and the ends of roots after lying in carbolic acid for four weeks had become so transparent as to be almost imperceptible when put under the microscope.

But we usually adopt means which produce immediate clari-

fication. It is a well-known fact that potassium hydroxide in a weak aqueous solution dissolves protoplasm, or at least changes it into a transparent, homogeneous mass.[21] It is evident that this can be employed as a clarifying medium and all the more so since it exercises a clarifying influence upon the cell walls.

Hanstein[22] was the first to employ potassium hydroxide to any great extent as a clarifying medium, and he did it in investigations of the merismatic tissue of the vegetative point and the development of the germ.

Delicate sections need to be immersed but a few moments in the weak alkali solution, washed out and put in glycerine where they become perfectly transparent. The glycerine, however, should not be used in a concentrated form but diluted with alcohol or water.[23] "Thicker sections require longer treatment with the potash solution and subsequent washing in hydrochloric or acetic acid and ammonia. The section may easily become too transparent so that the cell wall can no longer be recognized. By using a weak solution of alum they again become distinctly visible and we get in this way the best preparations" (Hanstein).

The manipulation in this method of Hanstein, which best commends itself on account of its general suitableness, is the following. The sections to be bleached are first treated to a weak solution of potassium hydroxide in a porcelain vessel or on a slide, for a few seconds, or even some minutes according to circumstances, and then carefully washed in distilled water. Hydrochloric or acetic acid is used as a neutralizer (sometimes one and sometimes the other), the section washed out again and then laid for a short time in weak liquid ammonia.

When a tissue that is to be clarified contains resin and fat masses along with protoplasmic substances, a method first suggested by Pfeffer,[24] for making it transparent, may be successfully

[21] Sachs, Lehrb., III Auflage, p. 42.

[22] Hanstein, Die Scheitelzellgruppe in Vegetationspunct der Phanerogamen (Festschrift die Niederrh. Ges. f. Natur.-u. Hielk z. Jubilaum der Univer. Bonn, 1868).—Hanstein, Die Entwicklung des Keimes der Monokotylen und Dikotylen (Bot. Abh. herausgegen v. Hanstein, Bd. I, Heft 1, 1870).

[23] Hanstein, Entw. d. Keimes, p. 5.

[24] Pfeffer. Die Entwicklung des Keimes der Gattung Selaginella (Botan. Abh. herausgegen v. Hanstein, Bd. I, Heft 4, p. 35).

employed. The preparation is laid for a short time in moderately concentrated potassium hydroxide. Wash this out imperfectly and repeatedly add absolute alcohol. A considerable quantity of the fat will be dissolved in it, as will also the resins and those substances which are produced by the effect of the alkali. The tissue which is much collapsed will often swell out again perfectly by the repeated addition of water, and especially so when the potassium hydroxide was not fully washed out with the water, since the potassium carbonate, which always exists in the reagent and is insoluble in alcohol, would be precipitated in the cells. The preparation should now be put in water containing a very little muriatic acid, and we have an object, which, if the effect of the alkali has been rightly regulated, leaves nothing to be desired.

Recently Russow[25] has recommended the so-called potassium alcohol as a bleaching medium. This is produced by mixing absolute alcohol with the concentrated solution of potassium hydroxide, the latter being added till a little precipitate is produced. It should be shaken frequently and left to stand twenty-four hours. The resulting pale yellow, clear fluid should be poured off from the settlings and must be diluted with distilled water before using (2 : 1). The potassic alcohol is applied in the same way as the potassium hydroxide solution in Hanstein's bleaching process. It is to be preferred to the aqueous alkali solution because the cell walls are not so much swollen in it as in that.

[A serviceable and easily made bleaching fluid is prepared by the following methods: To half a litre of distilled water add about 50 grammes of fresh chloride of lime. Shake thoroughly and while the lime is in partial suspension, add to it a saturated solution of common washing soda (carbonate of soda) until it becomes thick and turbid. Allow it to stand until thoroughly settled when the clear supernatant liquid should be drawn off with a siphon and kept in a well-stoppered bottle in a dark place.]

[What seems to be a practical, and, for delicate sections, a very desirable method of bleaching is proposed by Mr. Sylvester

[25] Mém. de l'Académie de St. Petersbourg, VIIe Sér., t. XIX, No. 1, p. 15.

Marsh.* Take two wide-mouthed bottles, holding each about 75 cc. Fit corks to them and connect them by a glass tube bent in such a way that it will pass very nearly to the bottom of one bottle but only just below the cork of the other. The cork having the long leg of the tube should either also be perforated in another place or a notch cut in its side, so as to permit a free passage of air from within. Now fill one bottle nearly full of distilled water into which put the sections to be bleached. Put in the cork with the long end of the tube. Cover the bottom of the other bottle with chlorate of potash in crystals and pour over it 5 cc. of strong hydrochloric acid and stop the bottle with the cork having the short end of the bent tube. The yellow vapor of chlorine (or euchlorine) evolved, immediately passes over by the tube into the water containing the sections. When the water becomes saturated the excess rises and escapes through the opening made in the cork. The escaping fumes may be got rid of by setting out of doors, on the outside ledge of the window frame for instance. When the bleaching is carried far enough—and it needs to be watched—the washing may be done automatically as Mr. Marsh proposes. Put the sections into a bottle, into the cork of which a small funnel is fitted, and which cork is also provided with a small orifice for the escape of the waste water. Now attach a small rubber tube to the lower end of the funnel which will reach to the bottom of the bottle. Put filtering paper into the funnel and set the bottle where a small stream of water may run into it. This is, of course, filtered and passes quite down to the bottom of the bottle and only escapes again when it has risen to the top. The washing may be done very thoroughly if one has time to wait for it. The advantages claimed for this method are that the sections are effectively bleached without being subjected to the destructive and disintegrating action of the chlorinated soda solution. And the sections will not suffer from a deposit upon them of a scum of carbonate of lime, as often happens when the common bleaching fluids are used.]

[E. Warming finds carbolic acid a valuable reagent in ren-

* Quoted from the *English Mechanic* by A. L. Woodward, in American Monthly Microscopical Journal, Jan., 1881, pp. 8, 9.

dering bacteria transparent. No doubt it would be found likewise serviceable with many other kinds of vegetable tissue, both for clarifying and for mounting. Its properties and relations to vegetable histology have, apparently, been but little studied.]

[*The Alcohol and Nitric Acid Method.** Place the sections in a watch-glass, add alcohol of 36° into which pour drop by drop, concentrated nitric acid until the red vapors of the hyponitric acid are disengaged. If the preparations are violently attacked cover the watch-glass with a bell glass and watch the process. As soon as the preparations rise to the surface of the mixture, raise the bell glass and by means of two wooden needles push them to the bottom of the liquid. When there is no disengagement of red vapor at the normal temperature, set fire to the alcohol in order to concentrate it further, and warm the watch-glass on a piece of wire gauze over a gas burner. Under these conditions the cell walls undergo a considerable thinning and all their contents disappear. They become so delicate that the difficulty is to remove them from the fluid in which they have been treated to the glycerine on the slide. This may be done, however, by adding to the still warm alcohol a little chloroform. This hardens the sections, when by means of wooden patula they may be transferred to the glycerine when they will again soften. This process is recommended for sections which are to be photographed.]

[*Chromic acid*, according to v. Höhnel, gives transparency to the cell walls of cork, epidermis and cuticular tissue and the envelopes of pollen grains.]

[*Calcium Chloride.* When it is desired to give transparency to the preparation without thinning it, especially if the tissue is very young, it is well to use the process followed by Treub† and afterwards by Flahant‡ and described by the latter as follows. Put the sections in a watch-glass, or in a small porcelain capsule with one or two drops of water. The drop is covered

* Recherches sur l'appareil tegumentaire des racines, Paris, 1881, L. Olivier. Quoted in Jour. Roy. Micros. Soc., Vol. III, No. 5, 1883, p. 744.

† Le méresteme primitif de la racine monocotyledones, Leyde, 1876.

‡ Recherches sur l'accroisement terminal de la racine chez les Phanerogames, Ann. Sc. Nat. VI (1878), p. 24. Quoted in the Jour. Roy. Micros. Soc., Vol. III, No. 5, p. 744.

with a little dry calcium chloride in powder, and slowly warmed over a small flame until the desiccation is nearly completed, and then withdraw it from the flame and add a few drops of water to dissolve the calcium chloride. The sections will now float on the water. They may now be placed in glycerine in which in a few hours they will be sufficiently transparent. This treatment does not dissolve the cell contents but darkens it by slightly thickening the original very thin walls. The walls become at the same time clear and brilliant. The opacity of the cell contents obstructs the sight if several layers are viewed at once. A. B. H.]

VI. PREPARATION OF MICROSCOPIC SPECIMENS OF FOSSIL PLANTS.[26]

Fossil plants occur in so many different ways that the methods of their preparation for use in microscopical investigations must be various. Sometimes indeed they occur in a condition such that they need no other preparation than do the like recent forms. They may be used either without further preparation, as microscopical objects, or they may be got ready by maceration on incineration, or by making thin sections. Since these manipulations have already been described in another place we shall confine ourselves here to giving illustrations of them by some examples of their application to the fossil flora.

In the first category among others belong the *Diatomaceæ*, which in many periods of the earth's development have occurred in such vast numbers of individuals — though of fewer species — that they really enter into the formation of the rocks. All those fossils which are designated polishing powder, tripoli, mountain meal, siliceous marl, etc., consist in by far the greater part of the siliceous frustules of bacillaria. These are sufficiently prepared for microscopical examination, when a little of the substance is taken up on a needle or lancet and placed upon a slide, moistened, and a cover-glass put over it.

A good example of the maceration process is afforded by peat

[26] Since I have had no experience in preparing microscopic specimens of fossil plants, my dear friend, Director Dr. Hugo Conwentz in Danzig, has kindly undertaken to prepare this section.

and stone coal. Göppert, as long ago as the year 1836, employed a method[27] by which vegetable remains could be detected in the densest varieties of coal. He treated the coal first with nitric acid, in order to prevent the potash salts melting together with the siliceous earth in heating. He then burned the coal and treated the ashes with acids. The residuum, when examined afterwards with the microscope, was found to contain more or less silicated, epidermal cells, tubes with simple and bordered pits, scaleform tissue, etc., either partly or wholly preserved. As Göppert succeeded in detecting plant cells in the compactest kind of coal, even anthracite from the graywack of Leibschütz in upper Silesia, what might we not naturally expect to obtain by a direct examination of the specimen itself? Very often ducts with alternating dots are found among the organic remains to which Göppert had already, in 1838, directed attention. They belong to a coniferous wood, *Arancarites carbonarius* G., which occupies an important place in the coal formation and almost exclusively furnishes the material for the "fibrous coal" of the mineralogist (mineralogical wood coal, Werner).

On the upper surface of this layer of coal the wood cells may be recognized by their fine striated velvety appearance, while in the interior they are invisible on account of the solid texture of the coal. In recent times Count Fr. Castracane[28] has found diatoms in Liverpool coal by the application of a method like that of Göppert. He pulverized it and at a red heat exposed it to a current of oxygen, and macerated the decarbonized powder according to the methods of Schultze already described above.[29] One can apply this method to many kinds of stone coal and peat. A portion of it should be pulverized and then boiled with a mixture of potassium chlorate and nitric acid, and then treated with water and dilute aqueous caustic ammonia, and at last with alcohol as long as any soluble material can be extracted from it.[30] When the residuum is examined with the microscope, isolated organic parts may be recognized, as fern spores, the remains of sporangia, etc.

[27] Göppert, Die fossilen Farnkräuter, Breslau und Bonn, 1836, p. XVIII.
[28] Pringsheim's Jahrb., für Wissensch., Botanik, Bd. X, p. 1, *ff.*
[29] See page 163.
[30] Verhandl., der Berliner Akademie der Wissenschaften, 1855.

Many of the bituminous woods embedded in the alluvium, diluvium, and in the tertiary strata are so well preserved that transverse and longitudinal sections may be made from them in the way usually adopted for recent woods. With others, on the contrary, it is recommended to moisten the cutting surface, not with water, but with a dilute solution of potash, to prevent the section from falling apart. In the same way we frequently obtain suitable sections of the large pieces of amber embedded in the wood. But in case the solidity of the wood has been at first disturbed, and the structure of it loosened, it should be embedded for half a day or longer in a thick solution of gum and then the desired section can be made. In most cases it will be well to treat the section to alcohol to clear it up.

In contradistinction to the above mentioned fossils, the great majority of such plants require a treatment differing entirely from this, namely, grinding smooth and grinding thin. To this group belong on the one side the series of fossil resins, especially amber, and on the other, woods which by various means have become petrified. When, for example, one obtains a piece of amber containing blossoms or parts of blossoms which he can examine with a low power he will only need to grind it to a flat surface. But if there is inclosed some form of fungus, pollen, or wood tissue which must be more strongly magnified the preparation of a thin section becomes indispensable. Petrified woods should be ground thin only when they can then be examined by transmitted light, that is to say, when they are petrified by such substances as in very thin sections possess a certain pellucidity. For example, woods which have been metamorphosed in marcasite, copper ore, etc., cannot be examined with transmitted light, but must be illuminated from above.

Most of the petrified woods—and there are in the different strata from the Devonian to the Oligocene, a very great number of them—require a special kind of preparation to make it possible to examine them with transmitted light. With many, particularly coniferous woods, it may be done by a skilfully struck blow, preferably in a radial direction, splitting off thin splinters, which, without further preparation, may be applied to the desired purpose. In order to increase this transparency

they may be inclosed in water or Canada balsam and placed under a cover-glass.

It is very difficult to split off a sufficiently thin splinter in a tangential direction, and in a horizontal it is quite impossible. Since a knowledge of the three named aspects of the wood is necessary in order to determine to what particular order it belongs, thin sections cannot very well be dispensed with. But in many cases, when the inquiry only seeks to determine if the wood be coniferous or deciduous, the test can be adequately made with the thin splinter. The preparation of ground sections in general was first made with fossil wood by W. Nicol,[31] and was then published by Witham in the "Observations of fossil vegetables, London, 1831." Independently of this Göppert and Unger in Germany invented separately a like method, by which they ground small splinters very thin, while hitherto petrified wood had been ground upon one side only, and had been examined with reflected light. This method was greatly modified by the authors themselves, as it has been by others till now scarcely two naturalists use exactly the same method throughout in the preparation of ground sections.

It should be incidentally remarked that the attempt was afterwards made to prepare ground sections of rocks in order to examine their structural relations with the microscope. This method was founded in 1858, by Sorby's treatise "On the microscopical structure of crystals indicating the origin of minerals and rocks,"[32] and in Germany Zirkel deserves the credit of having done good services in this direction by his "Microscopical studies of stone."[33] Through him and others, the microscopical investigation of rocks has been so far advanced that it has led to a complete transformation of petrography. The microscope has become to-day an indispensable instrument, not only for the botanist and zoologist, but for the geologist also.

The way and manner of preparing ground sections can be

[31] Further information concerning this subject can be found in the works named below. Zirkel, Die Mikros. Beschaffenheit der Mineralien und Gesteine, Leipsig, 1873.—Rosenbusch, Mikroskopische Physiographie der petrographische, wichtigen Mineralien, Stuttgart, 1873.—v. Lasaulx, Elemente der Petrographie, Bonn, 1875.

[32] Quarterly Journal of the Geological Society, London, Nov., 1858, Vol. XIV, p. 453, *ff.*

[33] Sitzb. d. k. k. Acad. in Wien, 1863, Bd. 47, Abth. 1, p. 226, *ff.*

varied in many ways, and we limit ourselves therefore to presenting the more general outlines of the subject.

1. *Cutting out the Specimen.* In order to be able to prepare a thin section it is first required to cut off the thinnest possible slice from the piece in hand. While it might be possible to knock off such a piece from a stone specimen, with a skilful blow of the hammer, it would be scarcely possible to do so with petrified wood, especially in a direction transverse to the original grain of the wood. On this account we adopt another method for obtaining the three different sections of fossil wood. We cut them off from the specimen. If the material has but little consistency it were better to saturate it previously with Canada balsam [first hardened and then softened by warm-

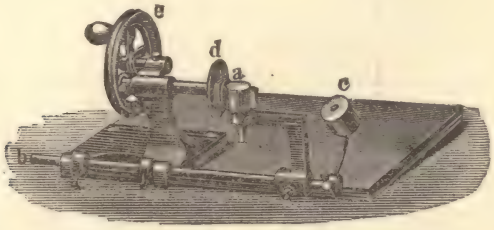


FIG. 95.

ing] in order the more easily and safely to take away sections of it which will hang together. Fig. 95 represents a small hand cutting-machine.* The specimen or a fragment of it is fastened to the iron holder *a*, by means of the common stone-grinder's cement. The holder rotates in two directions and is movable also on the axis *b*. A weight *c* fixed to the axis *b* presses the specimen against the steel disk *d* which is put in rotation by means of a small wheel *e* having a cogged rim. With this contrivance it is clear that one may cut thin pieces from the specimen, in different directions, without removing it from its fastening. The weight should not be too heavy, for if the fossil

* This, and the combined cutting and grinding machine, illustrated in Fig. 97, are made by Messrs. Voigt and Hochgesang of Göttingen, the former for 66 M.=$16, and the latter for 200 M.=$50. I am not informed what firm, if any, in this country, make or keep, for sale, these machines. Doubtless any of the principal optical houses would import them to order. A. B. H.

presses too hard against the cutting disk it may be caused to oscillate and break the thin slice of stone. Emery mixed with water should be used as a cutting medium. While turning the wheel with the right hand the other may be employed in putting the emery on the disk by means of a hair pencil. There is also arranged around the lower part of the disk, a box of sheet metal to catch the ground material and to prevent it also from being scattered about laterally. This was left off the drawing on account of its covering up essential parts of the apparatus. If one wishes he can so modify the construction of this machine as to propel the large wheel by foot-power like a sewing machine.

As to the size of the thin piece it is recommended to take a surface about 15 mm. square at the outset. It will be somewhat reduced in size afterwards, chiefly in consequence of grinding away the edges, so that when finished it will not, perhaps, be more than 12 mm. square, which generally is enough.

The next step is

2. *Grinding down the Specimen.* By this expression we understand grinding the surface smooth. If the preparation is large enough and of the right consistency it may be held in the hand, otherwise it should be cemented to a small glass plate which serves as a convenient handle. Canada balsam is a suitable cement. It should be sufficiently warmed to melt it, and the specimen should also be warmed over a spirit lamp or gas burner sufficiently to remove the moisture, then lay it on the balsam and again heat the plate till the balsam boils, being careful not to burn it. After the Canada balsam has cooled and the development of bubbles has ceased, press the specimen down fast. If bubbles yet appear under it, it will be necessary to repeat the process, else one may run the risk of getting the specimen broken.

An emery plate serves an excellent purpose for grinding down the preparation. It is first ground on a coarse grained plate and then the surface is polished on a finer one. Since the grinding stone must be kept moist, it may be laid in a suitable dish with a flat bottom and water enough poured in to cover the upper surface of the stone, Fig. 96. Should it not be possible to make the specimen sufficiently smooth on the fine grained stone,

it may be done on a ground-glass disk with emery which contains no coarse grains, or, better still, on a whetstone with the use of oil of turpentine.

These emery stones have but recently come into the market. Formerly the grinding was done on a cast-iron plate by means of emery and water. The new method has the advantage that it is simpler, leads more directly to the desired end, and makes greater cleanliness possible. According to the experience of others as well as my own, it may be commended as the best.

3. *Grinding the Preparation thin.* When one surface of the specimen has been ground and polished, it should be cleaned

FIG. 96.

with a hair pencil and plenty of water poured over it. Then it should be left in the air to dry. Do not under any circumstances undertake to hasten the process by rubbing with woollen or linen cloth, for this procedure unavoidably leaves small fibres upon the preparation which very much damage the microscopic image. It should then be separated from the glass plate by warming the Canada balsam and again cemented down, this time with the smooth side upon the plate,— first, however, having dried out any adhering moisture over a spirit lamp. Now begins the real thin-grinding, first again on a coarse grained emery plate, in doing which precautions are to be observed. The second surface is to be ground uniform and parallel with the first, but being careful not to grind away the edges. At last on the fine grained

stone when the preparation has become very thin, special care is to be taken not to grind the section through, or break it, although this does sometimes happen, even with the most skilful. In order to prevent the possibility of its occurrence, Zirkel recommended that four pieces of cover-glass be fastened to the four under corners of the glass plate, and of course the section cannot easily become thinner than those.

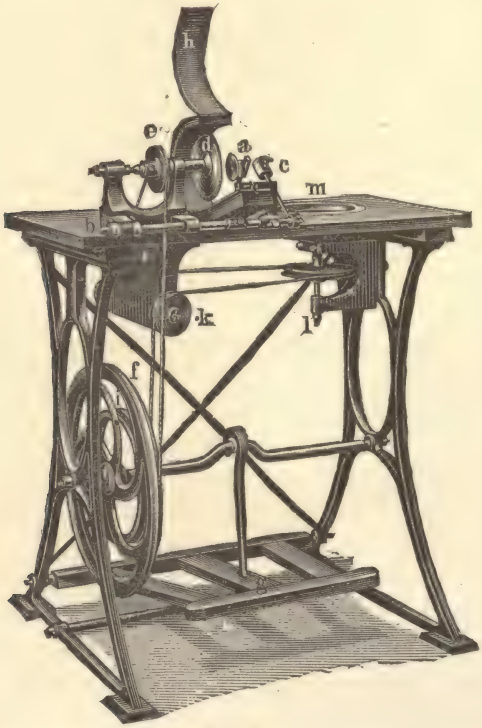


FIG. 97.

In respect to the thinness which the section should be made to attain, nothing in general can be determined, it depending rather in each individual case on the pellucidity of the material. If the material has a high degree of transparency, the preparation may be ground only relatively thin, in order to recognize clearly the structural relations. In other cases, when the material is almost entirely opaque, it is naturally recommended to grind

the section as thin as possible. In general one may consider a section sufficiently thin when one may see through it to read after having moistened it with water.

In order to save time and trouble one may fasten several specimens on the glass plate and grind them all at once. To do that, indeed, will require that they should be ground to a like thickness, and about equally withstand the action of the emery stone.

The process of preparing the section may be lightened, and time and care saved by the use of a machine which will rotate the disk while the specimen is held stationary instead of the other course as has now been shown. This is recommended by Rosenbusch and others. But, on the contrary, Zirkel in his extensive works does not approve of it. A larger machine has been constructed by the above mentioned firm to be driven by foot power and which can be used for both cutting and grinding the section. Fig. 97 gives an illustration of this combined machine. The cutting arrangement is essentially the same as described above; *a* is the carrier, *b* the movable axis for this and the weight *c*, *d* is the cutting disk, *e* a small driving wheel which is connected with the balance wheel *f*, by an endless band. The treadle *g* moves this wheel, *h* represents the folding guard box. The cast iron disk *m* by which the grinding is done runs on the vertical spindle *l*, in a depression in the table, by means of a belt from a second wheel *i* running over the guide pulley *k*. Probably this contrivance might be so modified as to allow an emery plate to be substituted for the iron one.

4. *Mounting the Preparations.* Since the glass plate is commonly larger and stronger than the object-slide used for the preservation of microscopic sections, and since also it is much ground and scratched by the process, the removal of the specimen when the grinding is finished is indispensable. This is often a matter of great difficulty and must be done by observing those precautions which are particularly set forth on page 197 of this work. When the specimen has been successfully embedded in Canada balsam, put on a cover-glass and warm the slide again carefully and press down the cover. After the specimen has become perfectly cold, the superfluous balsam should

be removed with a knife from about the edges of the cover and the slide perfectly cleaned with alcohol and water.

It still remains to consider the labelling of the specimen, which may be suitably done by strips of paper pasted on above and below as shown in the illustration, Fig. 98. On the upper one write the name of the fossil and designate in one corner the catalogue or other number, and in the other the direction in which the section is made, using the abbreviations H. for horizontal, R. for radial, and T. for tangential. On the lower label should be the rock formation to which it belongs, the locality, the date of finding, and the name of the collector. Besides this it is advised to write with a diamond, on the back of the slide opposite the label, some inscription by which the section can be identified with the specimen from which it is cut, if possible; for in case of the loss of the label the engraved note then becomes of the utmost importance. For purposes of identification, indeed, it is sufficient to engrave the catalogue number.

FIG. 98.

5. *Preservation of the Preparations.* Finally, the preservation of the specimens should be referred to, since it differs from the method applied to other microscopic sections. The latter, so far at least as they are mounted in a fluid, must be kept in a horizontal position, and, if desirable, provided with a protecting ledge. This is not required with ground-sections and so from this fact one may determine what suitable arrangements should be made. Commonly it is done in this way. The specimens are stored in wooden boxes provided with rack supports, whose distance apart corresponds to the size of the slide used.

I have recently contrived, for the preservation of microscopical ground-sections, for the West Prussia Provincial Museum in Danzig, a tray which rests upon a somewhat different principle. Its essential point is this, that the slide rests upon its short edge in a sloping position, thus making it much more convenient to read the label. Besides, one can thus store in one

tray, and have under his eye at once, a very great number of specimens. It is 42 × 46 cm. in size, and exactly fits into the drawers of those cases which are intended for microscopical, palæontological and geological collections. Two to four of these trays, according to the depth of the drawer, may be placed over each other. Thus from eight to sixteen hundred of these ground-sections may be opened for inspection in one drawer. The contrivance is illustrated in Fig. 99. A simplification of this plan might perhaps be made by substituting for each of the forty sloping transverse, cross ledges, in the two rows, two longitudinal ledges with sloping grooves cut in them.

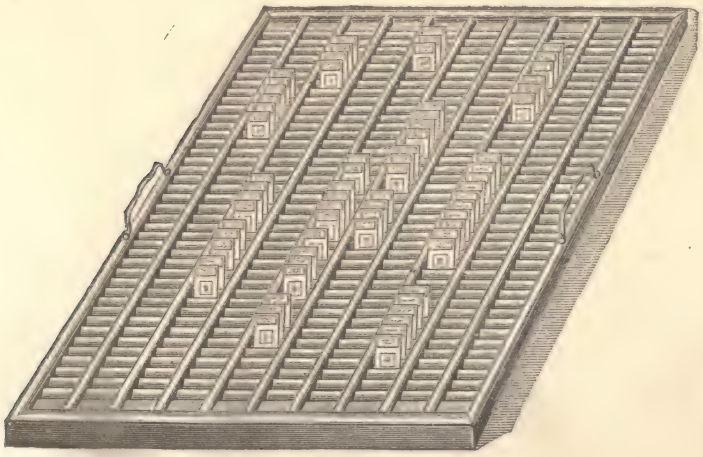


FIG. 99.

VII. THE PREPARATION OF PERMANENT MOUNTS.

Having already learned how an object should be prepared to be examined by the microscope, and having also particularly illustrated the different methods of preparing an object, we will here describe the preparation of permanent mounts. Permanent preparations are such as are preserved for a long time in a condition to be taken at any moment and subjected to examination.

1. Object-slide and Cover-glass.

It has already been several times mentioned that the preparation to be examined must lie upon a glass plate in fluid, and commonly be covered with a piece of thin glass. In the permanent preparation both glasses are always required. The under, stronger glass bears the name of the object-carrier (object-slide), the other, the thin plate, the cover-glass.

A, *The Object-slide*, is a plate made from pure mirror glass, often also, of common green window-glass. The most suitable thickness is from 1 to 1.5 mm. Glass thinner than 1 mm. is so thin as to be easily broken, and such as is over 2 mm. is too thick for this use. Perfectly colorless glass is much to be preferred, though glass with a slight green tinge may be used without harm. It is much more important that the slide, especially where the object lies, should be free of all minute air bubbles, flaws and scratches.

The edges should not be rough. They can be ground by the glazier without difficulty, or one can do it for himself on the whetstone with turpentine oil.

What form to give the slide is purely a matter of taste, only they should be neither too large nor too small. Those sizes which have come into most general use are the following:

(*a*) The English form, 76 mm. long × 26 mm. broad, Fig. 100, I.

(*b*) The German or Giessener society form, 48 × 28 mm., Fig. 100, I, II.

(*c*) The Vienna form, 65 × 25 mm., or 70 × 27 mm, Fig. 100, II.[34]

The form most used in Germany is form *b*. We prefer, however, form *a* to all others. [This is the one by far the most generally used in this country, only one firm offering for sale another size, 60 × 19 mm. A. B. H.]

Before using the slide it is necessary to clean it very carefully. This, in most cases, may be satisfactorily done by washing in

[34] Still other and less useful forms 75×30 mm., 70×35 mm., 70×30 mm.. 60×25 mm., 33×33 mm., the latter for stone sections, are also sold by opticians in Germany.

water and alcohol and rubbing dry with a clean cloth. For very dirty slides, or when one desires to have an absolutely clean surface, the following, although somewhat detailed, process is recommended. Lay the slide in a porcelain saucer with hot nitric acid. Take it out after a few minutes with the forceps and pour distilled water over it. Then for a short time

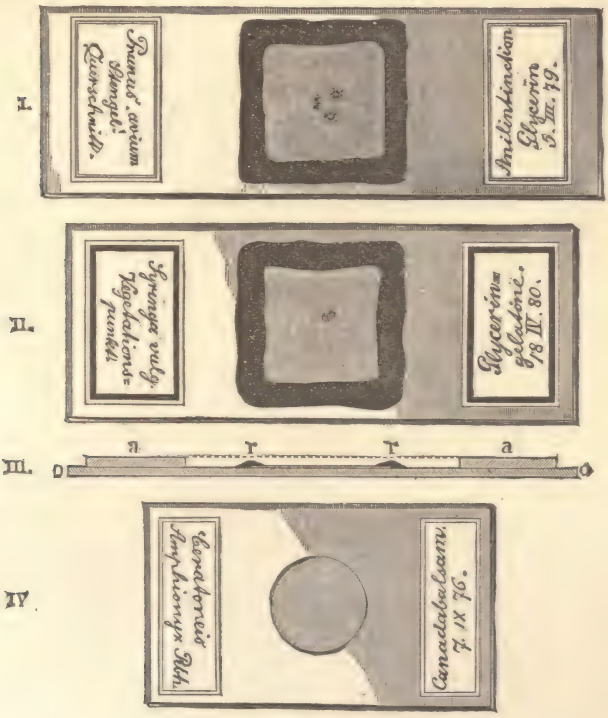


FIG. 100.

put it in a weak potash solution. Then wash with distilled water till the alkali is entirely removed. Pour over it absolute alcohol and finally cover it with ether and let this evaporate. By this process we get a perfectly clean surface, as bright as a mirror, without the use of a cloth.

B. The Cover-glass. The thickness of the cover-glass, as is well known, has its influence upon the microscopic image. For low and medium powers the cover-glass may be from 0.2 to

0.4 mm. thick, for still higher and the highest powers, the thickness should never be more than 0.15 to 0.10 mm., or still less than that.

The form of the cover-glass is in our judgment of considerable moment to the usefulness and durability of the preparation. There are circular, square, and rectangular oblong cover-glasses of various sizes.[35]

One should make it a rule never to use too small a cover-glass, either in the permanent preparations, or in those which are preserved only for present temporary use. Otherwise, in fresh preparations, one runs the risk of having the fluid employed exude around the cover-glass and come in contact with the objective, and in permanent mounts we find the cement ring around the edge of the glass seriously interfering with the focussing in the use of high powers. For common use squares of 18 mm. on the side, and circles of 15 mm. in diameter are perhaps best. We give the circles the preference before all others in mounting permanent preparations, since they are by far the easiest to cement perfectly tight. One naturally should employ the largest possible cover-glass in those investigations where reagents are used, which, if they come in contact with the objective are liable to injure it, or which may develop an injurious vapor upon it.

[For cleaning slides and cover-glasses, and for a convenient way to keep the latter so that they will be most easily accessible for immediate use, I know of nothing better than the plan suggested by Mr. C. E. Hanaman of Troy, N. Y., some years ago.* It is as follows:]

[A solution long in use by photographers for cleaning their negative plates and glass vessels is utilized, as being quite as efficacious as the nitric acid bath and wholly free from its disagreeable odors. The mixture consists of a cold, aqueous saturated solution of bichromate of potash to which is added about one-eighth of its bulk of strong sulphuric acid. The mixing should be made in a porcelain dish, or a thin glass vessel, as the

[35] In the price current of Stender the square ones are quoted of 10, 12, 15, and 18 mm. square, the round ones of 6, 8, 10, 12, 15, 18, 22 mm. in diameter, and the oblong, 26×21, 22×16, 18×12 mm.

* American Naturalist, Aug., 1878, p. 573.

heat generated might break a glass bottle. The vessel should be set outside for the liquid to cool, after which no more injurious vapors will be given off. Then the liquid may be kept for future use in a glass-stoppered bottle. The slides should be plunged one by one into a porcelain dish containing a quantity of the liquid, till all are put in, then tilt the dish in such a way as to cause the liquid to flow back and forth through the mass for a few moments, and then after pouring off the liquid place the dish under a stream of water from an open tap. They are then wiped dry with soft linen cloths.]

[The cover-glasses, after being treated with the cleaning liquid, are thoroughly washed with distilled or filtered water, and then taken out with the forceps one by one, and dried by laying each on one corner of a soft linen cloth on the table and gently rubbing, first one and then the other side with another part of the cloth. The cloths used for this purpose — preferably old, worn and soft ones — should be first boiled in carbonate of soda and rinsed in hot filtered or distilled water. In order to keep the covers clean, and make them most easily accessible for immediate use, as well as to greatly facilitate the selection of one of any desired thickness, the covers are finally arranged edge upwards, in a box or drawer, between strips of thick, white blotting-paper. The strips of blotting paper should be cut two-thirds as wide as the cover, should reach from side to side of the drawer or box, and should be separated at the ends by squares of the same paper, thus forming a rack in which the covers stand edge upward, and from which they can be readily picked out (Hanaman). A. B. H.]

2. Preserving Media (Mounting Fluids).

The freshly prepared botanical specimen, which is to be subjected to microscopical examination, is usually put in a drop of distilled water on the slide and a cover-glass put over it. But we have already seen that water is not the only fluid to be used in examining freshly prepared objects. So likewise in permanent mounts water is scarcely suitable to use, since most objects would in time decay in it. We must choose, therefore,

those preserving media which will keep the object from decay, and yet at the same time be sufficiently indifferent to it as not to change the object itself in the slightest degree. A large number of such media have been brought into use, but we shall undertake to enumerate only those deemed most important and most useful. They are either evaporating or non-evaporating fluids or substances, and those which may be applied in a fluid state but which afterwards stiffen.

A. Glycerine. For the botanist this is the most important of all the preserving fluids. It may be used with most vegetable preparations, but it preserves starch and chlorophyll grains relatively the best. In mounting the red algæ, bacteria and diatoms, it should not be used (Poulsen). Concentrated glycerine causes much shrinking of the tissue by withdrawing the water from it and a contraction of the primordial utricle. This, however, may be prevented in great part by first placing the preparation for a considerable time in a weak solution of glycerine and distilled water. Glycerine should be employed in mounting, either concentrated or diluted with water, in various proportions (1 : 1, etc.). Strong glycerine is often too powerfully clarifying to be suitable for certain kinds of tissue. It should then be diluted. Frey recommended the use of concentrated glycerine with the addition of a small quantity of pure carbolic acid. For other purposes he adds to 30 g. of glycerine, 2 drops of strong hydrochloric acid or, in place of this, concentrated acetic acid. Certain stainings require the addition of acid, otherwise the color will fade after a time. According to Dippel, the addition of a little acetic acid to concentrated glycerine modifies the effect of its shrinking properties, and its too powerful clarifying of thin tissue.

For the preservation of very delicate objects, green algæ, etc., a glycerine mixture invented by Hantsch[36] is recommended, which Dippel describes as follows: "Three parts pure 90 per cent alcohol with 2 parts water and 1 of glycerine. In order to moderate the effect of this fluid as much as possible at the beginning, the object is put in a drop of water on the slide to which a small drop of the mixture has been added. Then

[36] Hantsch in Reinicke's Beitragen zur neueren Mikroskopie, Heft III, p. 37, *f.*

the preparation is put in some place free from dust and allowed to stand till the water and alcohol have entirely evaporated. Then add another drop and let it evaporate as before, and continue this process till the object has as much glycerine as it should have to be properly mounted. In this way the good preservation of the object is perfectly assured. It is advised, however, before the mounting is finished, to let the object lie for some days in order to be fully convinced that the preserving medium has no more fluid in it that can be evaporated."

Objects to be mounted in glycerine should be first moistened in distilled water before they are put into the glycerine. Lay the object in a dish of water, put a drop of the glycerine on the slide and then transfer the object to it with the least possible amount of water adhering to it. Then put on the cover-glass. It will often be a matter of some difficulty with the beginner to know exactly how much of the glycerine to use in order to fill out the space under the cover-glass and yet not so much as to cause it to run out around the edges. The right quantity may very nearly be determined in the following way. A bottle with a long stopple, of the form of Fig. 101, is partly filled with glycerine, the stopper put in and lifted out: there will always be a drop of the same size hanging to its point. This is put on the slide as an experiment and covered with a glass of the size commonly used. Now, if there is too much or too little fluid on the slide, there should be some fluid taken out of or added to the glycerine in the bottle, as the case may be, till it stands at exactly the right place to give a drop of the required size. It should then be kept at the same height.

FIG. 101.

Glycerine should never be shaken because it thus collects small air bubbles which are transferred to the preparation. In order to free commercial glycerine from air bubbles, warm it a

little and then filter it through a common filter, or through fine glass wool directly into the glass bottle.

B. Glycerine-jelly. At the present time glycerine is being replaced by a fluid that contains glycerine, but which is used warm and stiffens on becoming cold,— the so-called glycerine-jelly. We believe that this medium is to be preferred in many cases to glycerine, since it is much more convenient to handle, and, as we have learned by careful experiments, it preserves many botanical objects in a very superior way, always assuming that good glycerine-jelly be used.

Kaiser[37] gave a method for preparing glycerine-jelly which we can certify from our own experience is very satisfactory. One part by weight of the finest French gelatine is soaked for about two hours in 6 parts by weight of distilled water. To this is added 7 parts of chemically pure glycerine, and to each 100 g. of the mixture add 1 g. carbolic acid. The mixture should then be warmed with constant stirring for ten or fifteen minutes, till all the flakes which were formed by stirring in the carbolic acid have disappeared. Finally filter while still warm, through glass wool, which has been previously washed and put in the funnel while still moist.

Glycerine-jelly stiffens perfectly in ordinary temperature, and so must be warmed each time it is used. For this purpose it should be kept in a thin walled test tube, so that it may be warmed in a moment. Then a drop is taken up by means of a glass rod and put on the slide, the slide itself being gently warmed, and the object which has been previously immersed in a weak solution of glycerine is embedded in it. Then the cover-glass (warmed) is put on and the whole left to cool. The preparation is completed when it afterwards has been provided with a ring of varnish or cement around the edge of the cover-glass.[38]

Nordstedt[39] in making glycerine-jelly dissolves 1 part of pure

[37] Kaiser in Botan. Centralbl., Bd. I. 1880. p. 25, *ff.* Vgl. further Brandt in Zeitsch., *f*, Mikroskopie Bd. II, 1880, p. 69, *ff.*— Poulsen, Botanisk mikrok., p. 42 (Translation Am. ed. p. 67).— Journal of the Royal Microscopical Soc., London. Vol. III, 1880, p. 502.

[38] Preparations, whose cell contents (protoplasm, chlorophyll, etc.) are to be preserved, must be hardened as much as possible, before they are mounted in glycerine jelly.

[39] Nordstedt: Om användandet af gelatinglycerine vid undersökning og preparering af Desmidieer (Botaniska Notiser, 1876, No. 2).—Poulsen, *l. c.*, p. 43 (Am. Trans., p. 67).

gelatine in 3 parts boiling water, and adds 4 parts of glycerine and (to prevent fungus growth) a piece of camphor.

A third method of making and using glycerine-jelly is given in the American Monthly Microscopical Journal[40] as follows: "The jelly is made by dissolving transparent isinglass in sufficient water so that it makes a stiff jelly when at the ordinary temperature of the room. When the slides are mounted, add one-tenth as much good glycerine, and a little solution of borax, carbolic acid, or camphor water. The mixture while hot should be well filtered through washed linen or other fabric, as it will not go through common filter paper, and the subsequent addition of a little alcohol improves its working. Objects, if perfectly clean, may be transferred from water directly to this medium which should be slightly warmed before using. The cover is adjusted* and the slide put away till a number have accumulated. The cover should not be pressed down too hard, and a liberal amount of jelly used to allow for shrinkage in drying. The slides may be finished as soon as the jelly has set, or may be left for several days. If air bubbles are entangled they will usually escape while drying, or they may be driven out by warming the slide a little. When ready to finish the slides, take them to a water cooler and let the ice-cold water drop over them, while, with a rather stiff camel's hair brush all the superfluous jelly may be washed away by aid of the flowing water which keeps the jelly under the cover hard. The slides are then dried with a towel, or cloth and finished with a ring of cement."

For the rest, mixtures of glycerine with gelatine, gum arabic, glue or other stiffening substances have been employed by various microscopists for a long time past. Schacht's glycerine mixture consisted of 1 part gelatine, 3 parts water, 4 parts glycerine. Deane mixed 4 parts glycerine with 2 of water and 1 of gelatine, the latter being dissolved in warm water and the glycerine added. Klebs recommended 2 parts concentrated isinglass solution with 1 of glycerine. Beale softens pure glue

[40] Vol. II, 1881, pp. 4, 5.

* The cover should always be adjusted with the slide upon the self-centering turn table, an apparatus to be described farther on, in such a way as to be exactly in its center. A. B. H.

in water, dissolves it in a glass vessel in a water bath, adds a like quantity of glycerine and filters through flannel.

C. Canada Balsam. In animal preparations this resin comes into most extensive use as a mounting medium, while in vegetable preparations it can seldom be used, because it exercises a quite too powerful clarifying effect on the cell walls of delicate sections, and most vegetable preparations containing water cannot bear the previous dehydrating necessary for mounting in Canada balsam. But it is principally applicable to the mounting of diatoms, hard seed coverings, spores, siliceous epidermal cells, and ground-sections of fossil plants.

We should use the best to be found in the market, having the clearness of water, and not too thick. Balsam which has become too thick by long keeping may be thinned by turpentine oil, chloroform or ether. In order to prevent evaporation as much as possible, it should be kept in wide-necked, glass-stoppered bottles. E. Kaiser has recently put into the market Canada balsam in tubes dissolved in spirits of turpentine. It is a very limpid, almost colorless fluid which is put up in metallic tubes like oil colors. The balsam in this form is very handy to manage, dries quickly and very seldom forms air bubbles in the preparation.

Mounting in balsam is done as follows: The object to be mounted must contain no trace of water, so it must be perfectly dried in a drying box (like diatoms), or—since all preparations will not bear this kind of drying—the water must be removed by putting the specimen in absolute alcohol from which it is transferred to turpentine oil, or oil of cloves. Now put a drop of the balsam on a slightly warmed slide, take the object from the fluid in which it is lying, let all run off that will, and then put it in the balsam, which should cover it on all sides and all over, the slide being kept warm meanwhile by being held over the flame of a small alcohol lamp [or over the short chimney of a small, low kerosene lamp. For all warming purposes in mounting, such a lamp is quite as good as an alcohol lamp, and naturally much less expensive to keep going. I always use one under my warming table in mounting with glycerine-jelly as well as with balsam. A. B. H.] For mounting in balsam as

well as in glycerine-jelly, it is best to use circular cover-glasses, as there is much less risk of having air bubbles than when using those with corners. Should they occur, however, under the cover-glass, they may be removed by leaving the slide in a warm place for a considerable time, in an inclined position, as on the iron plate of a stove. When the preparation is several days old the balsam will become hard, and all which has exuded about the edge of the glass may be scraped away with a knife, and the last traces of it removed with a cloth, and a little alcohol, or oil of turpentine. It is not absolutely necessary to put a varnish ring around the preparation; however, many microscopists take pains to do it.

[As a substitute for Canada balsam and other solutions of resinous gums, used as mounting fluids, I have for many years employed with great satisfaction a fluid made after the following formula.

(1) Gum mastic 38 g. dissolved in 55 cc. of chloroform.

(2) Gum dammar 38 g. dissolved in 55 cc. spirit of terebinth. Mix the two solutions and filter.

It may be employed not only as a mounting medium, but also as a temporary cement for inclosing glycerine mounts with which fluid it will not mingle at all or run in under the cover. Afterwards stronger cement should be applied over this to keep all secure. A. B. H.]

D. Chloride of Calcium Solution. Chloride of calcium is the oldest preserving medium known for permanent botanical preparations, it having been recommended by H. v. Mohl.[41] It was in earlier times almost the only preserving medium known. According to Harting, Dippel, Nägeli and Schwendener it serves an excellent purpose for cell-wall preparations, but not for chlorophyll and starch objects since these swell up in it and are quite destroyed. In recent times chloride of calcium has mostly gone out of fashion, and indeed not without good reason, for it is not easy to find a cement that will hold it. It is used either in a saturated solution or in different degrees of dilution (1 $Ca\,Cl_2$: 4—8 H_2O, Harting. 1 $Ca\,Cl_2$: 3 H_2O,

[41] Hugo v. Mohl, Mikrographie, p. 335.

Dippel), adding to the fluid some drops of chemically pure hydrochloric acid.

E. Other Preserving Fluids. Besides those already mentioned, there is a whole series of mounting fluids to which, however, but a passing word can be given, partly on account of their doubtful value and partly because they are suitable for but comparatively few preparations.

(*a*) *Sugar water*, with a little corrosive sublimate added to prevent the growth of fungi, is, according to Nägeli and Schwendener, suitable for all such objects as are too much changed by glycerine and calcium chloride.

(*b*) *Solution of Corrosive Sublimate.* Of this, numerous compounds have been made and recommended, first, that of Goadby (Goadby's Fluid):

Sodium chloride	120.00 g.
Alum	60 00 "
Corrosive sublimate	0.25 "
Boiling distilled water	2.33 l.

Pacini modifies the mixture as follows:

I		II	
Corrosive sublimate	1 part	Corrosive sublimate	1 part
Common salt	2 "	Acetic acid	2 "
Glycerine	13 "	Glycerine	43 "
Distilled water	113 "	Distilled water	275 "

The mixture is left to stand for two months, and is then diluted one part thereof with three of distilled water and filtered. The botanist, however, finds little use for compositions of corrosive sublimate.

(*c*) *Creosote Mixture.* Harting recommends for some preparations an aqueous solution of creosote, consisting of a saturated solution of creosote in 20 parts of water, to which 1 part of 30 per cent alcohol is added. Beale gives the following formula for a complicated mixture. Mix 180 g. methyl alcohol with 11 g. creosote, then add enough pulverized chalk to form a thick pulp; then, in a mortar, slowly add with constant trituration, 1920 g. water and put in a few pieces of camphor. After two or three weeks, filter and keep the filtrate in a well closed glass bottle. It is a good preservative for desmids.

(*d*) *Topping's Fluid* consists of one part absolute alcohol and five parts water, or in place of the water, 4 parts water and 1 part acetate of alum. An equal volume of glycerine is added to the mixture and should be used principally in preserving objects stained with carmine.

(*e*) *Potassium Acetate.* This salt was first recommended by Sanio,[42] and was afterwards designated as a good preserving medium by Dippel. It preserves the chlorophyll beautifully without a sign of shrinking of the cell wall. Use a saturated solution in distilled water. It is employed as a preservative for bacteria which have been stained with aniline.[43]

(*f*) *Preserving Medium for Algæ.* For preserving confervæ, and related forms, including desmids (fresh-water algæ), P. Petit[44] employs the following composition which is filtered after solution:

Camphor water	50.00 g.
Distilled water	50.00 "
Glacial acetic acid	00.50 "
Crystal copper chloride	00.20 "
" " copper nitrate	00.20 " *

(*g*) [*King's Fluid for preserving Marine Algæ.* Rev. J. D. King finds the following composition entirely satisfactory for mounting and preserving microscopic specimens of marine algæ. Powdered alum 62.20 g., corrosive sublimate 0.258 g., dissolved in 2.27 l. of pure sea water and filtered. For several years past I have made much use of a mixture of sea water and glycerine for this purpose. It may be mixed in various proportions, though 5 parts of the former to 1 of the latter, will be found to do well in most cases. It is a good plan to filter the sea water at first and then let it stand tightly corked in a glass bottle for several months, then filter again and mix. A. B. H.]

[42] Sanio in Botan. Zeitg. 1863, p. 359.

[43] Poulsen, *l. c.*, p. 44 (Translation, p. 69).

[44] Brebissonia, Jahrg. III, 1880, p. 92.—See also Cornu et Rivet, Des préparations microscopiques, Paris, 1872.— Concerning a peculiar method of preparing for examination marine algæ, which have been once dried, see C. F. Jones in Northern Microscopist, Vol. I, p. 54-56. Also Jour. Roy. Microscop. Soc., London, Vol. I., Series II, 1881, p. 530.

* Mr. G. W. Morehouse adds to this 100 g. strong glycerine, or if the specific gravity is too high, less glycerine. See Am. Monthly Microscopical Journal, Dec., 1883, p. 234. A. B. H.

(*h*) *Monobrom-Naphthaline*, according to the recent experiments of Abbe and Dippel, is a suitable mounting medium for objects which are to be examined with very high powers, as, for example, diatoms used as test objects.

(*j*) [*Styrax and Liquid Amber.* Dr. H. Van Heurck* has published an account of his very satisfactory experience with these resins, *Liquidamber orientalis* Mill, and *Liquidamber styraciflua* L. as a mounting medium for diatoms, and other such objects requiring a medium of a high index of refraction. In this case it is 1.65, Canada balsam, which this is intended to take the place of, being 1.53. The commercial article is first dissolved in chloroform and filtered to purify it from the granular substances which it contains,—and the solution thus obtained is used in the same manner as a like solution of Canada balsam. It will not form bubbles of air in heating. It is recommended to expose the liquid amber in a thin layer to the direct light of the sun for several weeks. This will cause it to discharge all of its water and most of its color. It becomes hard and then may be dissolved as before directed in chloroform. It may also be dissolved in benzine or a mixture of benzine and absolute alcohol. A. B. H.]†

(*k*) *Colored Mounting Fluid.* A complicated preserving medium, especially adapted for mounting starch grains, has recently been given by Seiler.[45] He describes its preparation and use as follows:

"It is necessary first to have some aniline blue staining fluid which we make after the formula given by Beale:

Soluble aniline blue	0.032 g.
Distilled water	31. cc.
Alcohol	25 drops.

A mixture is made of equal parts of glycerine and water, say 15 cc. each, to which are added 2 or 3 drops acetic acid. To this mixture of slightly acidulated dilute glycerine is added the

* Bull. de la Soc. Belge de Microscopie. Quoted in Am. Month. Micro. Journal, Apr., 1884, p. 69, 70.

† Prof. H. L. Smith has discovered two media for the same purpose, the nature of which he has not yet chosen to make public, which have an index of refraction respectively of 2.00 and 2.25. Sec. J. D. Cox. in Am. Monthly Micros. Journal, Apr., 1884, p. 71.

[45] Seiler's Compend. of Micro. Technology, Phil., 1881, p. 13, *f*.

aniline blue staining fluid until the whole mixture is of a decided blue color. A drop of this mixture is placed on a glass slide and some of the starch to be mounted is dusted over the top. The dusting can be done to the very best advantage by touching the starch with a camel's hair brush and then slightly shaking the brush over the drop of colored glycerine. The starch soon sinks to the bottom of the mixture and the cover is applied. With this method of introducing the starch, air bubbles are avoided. The cover is pressed down quite firmly upon the slide and the excess of glycerine carefully removed. The slide is then transferred to the turn table and a thin layer of dammar or balsam in benzole placed around the border of the cover. This soon hardens, and in a day or two the slide may be finished with a ring of white zinc, Brunswick black or other cement. The effect is this: the grains themselves have not taken the staining in the least, neither will they ever take it; they retain their natural appearance surrounded everywhere by the blue glycerine and the effect is most beautiful."

(*l*) [Mr. Karl Castelhun of Newburyport, finds the following very satisfactory for preserving sections of lichens:

Glycerine	7	parts
Alcohol	1	"
Water	6	"]

(*m*) [*Strong carbolic acid* is highly recommended for preserving vegetable tissue. It should be used with only so much water added as will keep it from crystallizing. "One great advantage of its use is found in the readiness with which it penetrates a specimen and mixes with the fluids used in mounting, such as water, glycerine and Canada balsam.*]

(*n*) [*Preservative for Fungi.* Thoroughly mix 1.134 l. white wine vinegar with 127.5 g. common salt and 141.7 g. pulverized alum and keep in a wide-mouthed glass jar. Put the fresh specimens of fungus into it. From time to time the liquid may be strained to take out impurities.†]

* Wm. J. Pow, in Am. Month. Micro. Journal, Jan., 1883, p. 8.
† Mary E. Banning in Bulletin Torrey Botan. Club, Dec., 1882, p. 153.

(*o*) [*Wickersheimer's fluid* for preserving algæ, lichens, fungi, etc., preserves the color of most delicate structures quite perfectly.

Alum	100 g.
Common salt	25 "
Saltpeter	12 "
Carbonate of potash	60 "
White arsenic	20 "

Dissolve by boiling in 3000 cc. of water. Cool and filter and add 1550 g. glycerine and 300 g. methyl alcohol (wood spirits).*]

[This fluid is an excellent preservative for all kinds of land plants; and plants which ordinarily become stiff and brittle by drying will always retain their natural flexibility if, previously to drying they are immersed for a little time in this fluid, till they become saturated with it. A. B. H.]

Besides the preserving and mounting fluids here mentioned, there are a large number of others, formulæ for which are scattered through the different works. We have not included them in this work because the greater part of them have been used only by their inventors, their usefulness not having been tested by others.

VIII. MOUNTING THE PREPARATION IN PRESERVING FLUID.

We have already pointed out how to proceed in putting the specimen into a preserving medium such as glycerine-jelly and Canada balsam. This is not usually attended with much difficulty and the beginner soon learns to do it successfully after a few experiments.

It is otherwise in mounting with fluids. In doing this we have carefully to measure out the exact quantity required to fill the space between the glass cover and the slide, and then we must hermetically seal up this fluid in which the preparation is immersed, of the method of doing which we shall speak hereafter.

* Furnished me by Dr. L. Schöney of New York City. A. B. H.]

The little knack by which we may take out each time very nearly the quantity of fluid we need has already been indicated in the case of glycerine (see p. 219). The fluid may be prevented from running out about the edges of the cover-glass by putting a thin rim of varnish, hereafter to be described, upon the slide which is somewhat smaller than the cover-glass itself. Schacht, who invented this process, drew two varnish ledges on the slide corresponding to the two opposite sides of the square cover-glass; Dippel[46], three which formed a square with one open side, and Nägeli[47] added still a fourth thus closing up the rim of varnish. Each of these authors held his method to be the best. We leave it to the experienced worker to decide for himself between them. The round cells made by means of the turn table are very pretty, but one must naturally use the round cover-glasses with them.

Whether or not the slide be provided with a foundation ring, the process of putting the specimen in the fluid is the same. The slide is first breathed upon so that the fluid will readily adhere to it and then a sufficient quantity is laid on, and the section, which has been previously freed from air, is put into it. The slide is put under the mounting microscope and by means of a couple of needles the object is carefully arranged. In doing this it is well to press the section down gently till it adheres to the surface of the slide, so that in putting on the cover-glass one would run no risk of pushing it out of place. [To avoid this danger and the consequent trouble caused by thrusting the preparation from its place, Rev. J. D. King recommends the following method where the preparation is to be mounted in glycerine or any of its compounds. Put a thin film of glycerine-jelly on the slide where the object is to lie, and with the warm slide and the melted jelly arrange the preparation where it is wanted. Let the jelly cool and stiffen, then put on the glycerine, remove all the air bubbles under the mounting microscope, put on the cover and press it down expelling all the superfluous glycerine, seal and cement in the usual way. Then to render the fluid in which the preparation is mounted perfectly

[46] Dippel, *l. c.*, Bd. I. p. 474.
[47] Nägeli u. Schwendener, *l. c.*, p. 297.

homogeneous, warm the slide gently till the glycerine-jelly film is melted, when it will mingle freely and perfectly with the other fluid. This process is especially convenient where one wishes to arrange several small sections under one cover-glass. A. B. H.]

The following manipulation, putting on the cover-glass, must first be taught to beginners who often fail. If angular covers are used, he should take them up in the forceps by one corner, and having breathed upon the side which will come next to the glycerine or other fluid, he puts down the edge opposite to the forceps. Now he lowers the forceps till the drop of mounting fluid touches the middle of the glass. Now, when the skilful manipulator suddenly lets go of the cover-glass it falls into its place so that its edges lie parallel with those of the slide, and the mounting fluid is evenly spread out between the two glasses and there are no air bubbles. If we use slides which have a ring or square of cement already laid on them and put the edge of the cover-glass upon that while it is yet sticky, it will be fixed at once in its place, which is a matter of great importance in the subsequent hermetical sealing of the cell.

The cover being now successfully laid on, we examine the object with a magnifying glass or the preparing microscope, to see if it is still in its place in the middle of the cover-glass, or has been pushed aside. In the latter case (if we have not mounted the object in a closed cement ring as above described), we may replace it again by means of a common hair held between the thumb and finger and thrust in between the cover-glass and slide, or by means of a very fine glass thread which one may make by drawing out a piece of glass tube over the flame.

Very delicate preparations, as, for example, sections of the very young parts of flowers, "growing points," etc., are so delicate that the weight of the cover-glass will quite destroy them especially when to this is added a little pressure by the drying of the cement ring which holds the cover. There are several ways of preventing this. One proposed by Purkinje[48]

[48] Purkinje in Wagner's Handbuch der Physiologie, Artikel Mikroskop.

and Hugo v. Mohl[49] consists of laying small wax balls between the cover-glass, which by gentle pressure on the latter can be reduced to the exact thickness of the preparation. This method is in fact a very good one. In extraordinarily thin and delicate sections small pieces of the fibres of glass wool, or of the hair from the head, may be substituted for the wax balls to give the cover-glass the right position.

But, on the other hand, if the preparation is very thick, a little trough or cell about the height of the thickness of the section must be built up upon the slide, in which it may lie. For this purpose we build up a wall of varnish or shellac, of the size and shape of the cover-glass, by putting on one layer after another, letting each layer dry before adding another. We may also use wax instead of varnish, and then our cell will soon be done for that rapidly stiffens. But the wax in thick layers is always brittle and will not without injury bear sudden changes of temperature. On this account the cement cells are greatly to be preferred.

[Making cement cells by the use of the self-centering (or any other) turn table, is a matter of very little difficulty, but of the greatest importance to the microscopist. For all but the very deepest cells they answer the purpose perfectly, and for these metallic or glass rings may be used.]

[The strongest cement is the best for cells. Usually some solution or compound of shellac is preferred, directions for making which will be found below. Probably nothing better of this kind can be had than King's amber cement, or white cement. The slide is laid upon the turn table and concentrically clamped. A small hair pencil is dipped in the cement and while the turn table is being somewhat rapidly rotated the brush is carefully brought down upon the rotating slide so as to draw a circular band of cement 3 or 4 mm. wide upon it in such a position that, when the cover-glass is put on, its edge will come about in the middle of the band.]

[There are two ways of completing the circular wall of the cement cell. The one is to lay on the first coat of cement so carefully that the circle will have exactly the diameter and

[49] H. v. Mohl, Mikrographie, p. 328, *ff.*

breadth required in the cell. Let this thoroughly dry. Then put on another coat very carefully, exactly on the top of the first. Let it dry and again repeat the operation till the ring is built up high enough. When all is done and the last layer is quite dry and hard, bring the top of the ring to a flat and even surface with a smooth file, or upon a stone. It then may be laid away till wanted.]

[The other, and perhaps the preferable because the more rapid, way of making cement cells, is to lay on a considerable quantity of the cement at the first, making the inner edge of the circle come as near as possible to the position where it is wanted, but permitting the outer edge to spread out wider than the ring is to be when finished, making it 6 or 7 mm. broad if necessary, and lay on all the cement needful to finish the cell. Then with the point of a knife applied to the slide at the outer edge of the cement ring, while the turn table is rapidly rotating, turn the edge of the ring slowly inward narrowing the band and heaping up the cement until the desired height and breadth of the ring is attained. If the inner edge of the ring should need to be turned and smoothed a little, it may be done in the same way, but it would not be best to move it very far, for unless the work is very nicely done traces of the cement will be found on the slide at the bottom of the cell. If this should happen, the best way to clean it is to let it dry thoroughly, put it again on the turn table, rapidly rotate it, and with the smooth point of the knife turn off or scrape up the adhering traces of cement. The particles may then be removed with a dry cloth or camel's-hair brush. The top of the ring, when perfectly dry, should be made smooth and flat as in the other case. A. B. H.]

But if we wish to make a durable trough quickly, we shall have recourse to the so-called glass cell. It is a perforated glass plate, represented in Fig. 102, I, and is prepared from glass 0.5 to 1.0 mm. thick, rough ground on the underside, and thoroughly cemented to the slide. [Marine glue is excellent for this, perhaps the best, but the cements mentioned above will answer all purposes.] One can make glass cells for himself without much trouble. Taking a glass tube of about 12 mm. interior diameter and a thickness of wall of about 3 mm., have

it sawed up, at a glass grinder's into rings .5 to 1.0 mm. thick, Fig. 102, II. Then fasten them upon a glass plate with Canada balsam in much the same way as fossil wood is prepared for grinding, then with emery or with turpentine oil on a whetstone grind it flat and smooth. Then turn it over, recement it, clean away the Canada balsam and grind the other side in the same way. A still simpler way is to take glass strips 3 mm. broad, 1 mm. thick and of sufficient length, and by holding them in the flame of a Bunsen burner, bend them into the form given in Fig. 102, III, welding it finally at *a*. The joint should be made in the middle of the side rather than at the corner.

FIG. 102.

In mounting large specimens in shellac, wax, or glass cells the process is as follows. The cell is filled full of the mounting fluid, for example glycerine, and the specimen carefully laid in. When the cover is laid on, it should be fixed at one corner with a small drop of wax or shellac, which should be allowed to stiffen or harden as the case may be. But if some of the fluid has run out and got on the cover-glass it must be carefully removed, a matter sometimes of no little difficulty and labor. The first step toward cementing the cover-glass should be taken only when it and the upper surface of the cell are perfectly clean and dry.

It may be mentioned in passing, that this method of mounting especially commends itself for those slippery algæ which, when we undertake to mount them without a cell, directly we put on a cover-glass slip out from under its edge. In this way only have we succeeded in mounting the gelatinous fresh-water alga *Batrachospernum moniliforme*, after all other attempts to confine it had failed.

IX. CEMENTING AND FINISHING THE MOUNT.

When the preparation has been embedded in the preserving medium and the cover-glass laid on, the next step is to surround the edge of it with a border of varnish or cement, which when dry will fasten it to the slide, solidly and permanently, hermetically sealing up the preparation (see Fig. 100, I, II). Before, however, we describe this process, we should become acquainted with the nature of the cements or varnishes used in it.

1. Cements.

1. *Wax.* This is used in the form of a little wax candle, the wick of which is lighted and then, after a moment, when the wax is melted around it, we may dip a camel's-hair brush into it and immediately draw the wax rim on the slide around the cover-glass.

2. *Asphalt Varnish* (Brunswick Black). This consists of a solution of asphalt in like parts of turpentine and linseed oil. It can be had in any drug shop. For microscopical purposes the best only should be used. Really good asphalt varnish renders the very best service in cementing microscopical preparations notwithstanding Frey's assertion to the contrary. If it gets too stiff it may be thinned with oil of turpentine.

3. *Mastic Varnish.* I am not acquainted with the composition of this varnish. The dissolving medium is alcohol. It was first recommended for our purposes by Schacht. The microscopist's varnish of E. Kaiser appears to be like it, which from my own experience I can recommend. Both kinds when they become too stiff may be thinned with absolute alcohol.[50]

4. *Shellac and Sealing-wax Cements.* Dr. O. E. R. Zimmermann has kindly furnished me with the formula of a very useful shellac cement. "Dissolve good brown shellac in absolute alcohol, add aniline green and filter. The filtrate should now be allowed to stand protected from dust near a stove in a

[50] Frey, *l. c.*, p. 143.—Dippel, *l. c.*, Bd. I, p. 473.

wide-necked glass bottle till it has become so thick that it will not run when put on a slide with a hair pencil, but makes a sharply defined outline. This varnish never cracks off. When perfectly dry and subjected to frequent changes of temperature, isolated wrinkles will sometimes come in it, but it never loosens up so as to injure the object."

Thiersch[51] has given a formula for making a thin shellac cement which can be used with balsam mounts. Thick brown shellac cement (prepared by the solution of shellac in alcohol) is evaporated to the consistency of thin mucilage and colored with a concentrated solution of aniline blue or gamboge in absolute alcohol. To 60 g. add at last 2.5 g. castor oil, evaporate still a little further and keep in a well-closed bottle. If it becomes gradually too much concentrated it may be thinned with a few drops of alcohol.

Poulsen[52] gives a recipe for a third kind of shellac cement which he names the Gram-Rützou cement: "50 g. Canada balsam, 50 g. shellac, 50 g. absolute alcohol, and 100 g. ether are mixed and evaporated in the water bath to a thick syrupy consistency.

An alcoholic solution of sealing wax is often proposed in place of the shellac cements, but I have no great confidence in it.

[I am indebted to the kindness of Rev. J. D. King for two formulæ, one of a very excellent cement and the other of a "finish" which for its cementing power and its good appearance can scarcely be equalled, I think, by anything yet offered in this line. They are both compounds of shellac.]

[*King's Amber Cement* is made as follows. (1) Dissolve 453 g. of best bleached shellac in half a litre of 95 per cent alcohol. (2) In another vessel dissolve 1 part gum mastic in two parts alcohol and let it stand till perfectly clear. To the shellac solution; (1) add 38 g. of the mastic solution, (2) color with "dragon's blood" dissolved in alcohol and filter. Place it in the water bath and stir frequently till it comes to a boil. Filter through flannel, after which, if too thick, bring to a right consistency by means of strong alcohol.]

[51] Frey, *l. c.*, p. 143.
[52] Poulsen, Botanisk Mikrokemi. Translation, p. 71.

[*King's White Cement* is made in the same way omitting only the "dragon's blood."]

[*King's Lacquer Finish.** (1) Dissolve 453 g. Dennison's excelsior sealing wax in one-half litre or more, if necessary, of alcohol. (2) Dissolve 1 part best bleached shellac in 2 parts 95 per cent alcohol. To every 38 g. of No. 1 add 5 g. of No. 2 and 5 g. of Brown's rubber cement. Let it stand two weeks or more in a warm place, stirring it occasionally. If too thick to flow freely reduce with alcohol.]

[The color of this finish will depend of course upon the color of the sealing wax used, and one can thus exercise his taste in ornamenting his slides, at the same time that he secures in the best possible way the permanent safety of his preparations. A. B. H.]

5. *Copal Varnish* can be employed as a cement in connection with wax and asphalt varnish. I prepare it in this way. I put 5 g. of pulverized copal in a glass retort and pour over it 5 cc. each of absolute alcohol and oil of turpentine and 1 cc. of ether and carefully, slowly and gently warm until the copal is dissolved. Close the vessel, set it off and pour the clear, transparent varnish into a well closed glass-stoppered bottle.

6. *Dammar Varnish* used in connection with the foregoing is prepared in the following way. The best coarse grained dammar gum should be warmed a long time till all the water is driven out, then pour over it three times its weight of turpentine oil and when dissolved decant the clear, colorless varnish into a glass-stoppered bottle.

7. *Gold-size*, a cement much used by the English is prepared, according to Beale, in the following way. Twenty-five parts of linseed oil are boiled three hours with 1 part vermillion and $\frac{1}{3}$ part umber. The clear liquid is then poured off and like parts of well ground white lead and yellow ochre are slowly and gradually mixed in with constant stirring, further boiled and finally turned off and kept in a bottle for use.

* These cements and finishes may be had ready made of Rev. J. D. King, Cottage City, Mass.

2. Cementing Angular Cover-glasses.

[Inasmuch as this form of cover-glass is very little used in this country in botanical work, I shall condense what the author has to say upon it into as little space as possible. A. B. H.]

The tools to be used are small artists' hair pencils of the form represented in Figs. 103 and 104, the flat one for laying on the cement band and the smaller round one for touching up and finishing off the work, adding a little of the cement here and there. They should be kept scrupulously clean and the cement should never be allowed to dry or harden in them. This may be prevented by having little bottles partly filled with the solvent of the cement in which the brush is used, alcohol, turpentine, ether, etc.; and, having a hole though the cork, put the handle of the brush in it, letting the brush dip into the liquid and there to remain when not in use. The brushes may also be cleaned after each using with the cement solvent, and left dry.

Figs. 103 & 104.

If, in placing the cover, a small drop of the mounting fluid, glycerine, etc., runs out upon the slide it should be cleaned off before cementing. A hair pencil saturated with oil of turpentine will do this, and the traces of turpentine may be washed away with a brush dipped in ether.

When a foundation cell has been made and the cover-glass pressed down upon the soft cement in mounting so as to stick fast all around, the subsequent cementing and finishing are an easy matter. It is only necessary then to fill the flat brush with the cement and draw it slowly along the edge of the cover-glass, half on that and half on the slide, making the layer not more than 3 or 4 mm. wide altogether. Begin at the corner and go the length of the side at a stroke. So all around. The cement or varnish for the first coat should be of as thick a consistency as can be conveniently managed with the brush. The slide should be laid aside for a day or two for the varnish to dry;

then examine it with a magnifying glass to see if it is all tight; if not, apply more thick varnish. If the first coat is not substantial enough, apply a second of a varnish of thinner consistency, making the band extend a little beyond the edges of the first one. After a fortnight, even a third coating may be put on.

If no foundation cell has been made and the cover-glass has nothing to stay it but the adhesion of the mounting fluid, a drop of thick varnish should be put at each corner and allowed to harden, then the varnish applied as before, care being taken to use pretty thick varnish so that it will not run in under the cover and spoil the specimen. [If, with glycerine as a mounting fluid, a chloroform or benzole solution of Canada balsam or dammar, or the mastic and dammar solution described on p. 223 be used for a first coat, there will be no danger of its running under the cover, and one will find it very convenient to use one or the other of these with all glycerine mounts whether with square or circular glasses. A. B. H.]

Several coats of the finishing cement should be applied one after the other as they become dry.

Another method is to make the first layer of wax. The wick of a small wax candle is heated over the flame till it is thoroughly saturated with the melted wax and then drawn carefully along the edges of the cover-glass. It immediately stiffens and the cement may be applied at once. I usually put over a thin layer of wax a layer of copal varnish which very quickly dries, and then over that a third of dammar varnish which dries very slowly but is very durable. When it is dry I put on at intervals three layers of asphalt. Balsam and gelatine preparations should have one or two coats of asphalt. Thiersch's shellac cement may be used with the former, after a previous layer of Canada balsam dissolved in chloroform has been applied. [For all finishing processes I know of nothing better than Brown's rubber cement, or King's lacquer finish, the latter being the stronger and therefore the better. The white zinc cement so extensively used in this country is bitterly complained of by some and highly recommended by others. There seems to be no way of accounting for such marked differences of experience and opinion. It has served me well. A. B. H.]

3. Mounting with Circular Cover-glasses.

The use of circular cover-glasses is very much to be preferred. They are easier to cement, and are more secure, not having the weak points, the corners of the square ones, and it requires much less time to cement them, and they also have a much more elegant appearance than the angular ones.

[Mounting with and cementing circular covers requires the use of a turn-table. Those with a device for self-centering are so much better than those without that I would recommend no one to get any other.]

[Two forms are herewith represented in Figs. 105 and 106. The construction of them is so obvious that a detailed description will not be necessary. The circular brass plate, Fig. 105,

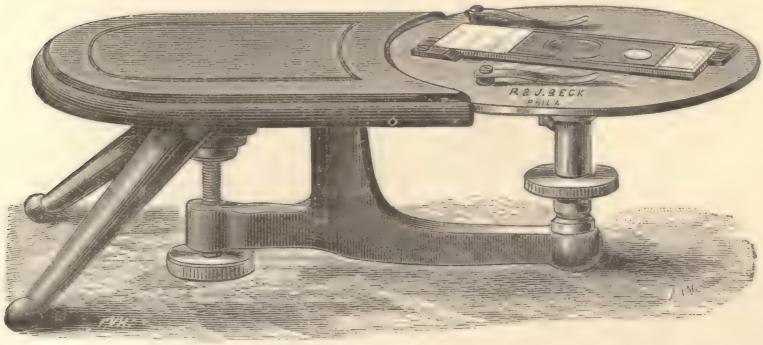


Fig. 105.

revolves on a central pivot smoothly, and with the least possible friction. It is actuated by a stroke of the hand along its milled margin. The self-centering apparatus consists of two rectangular jaws upon the upper surface of the plate which are made to clasp the slide at its diagonal corners. When the slide is held by these jaws it is concentric with the plate and with its axis of motion. The jaws are moved toward each other by a spiral spring beneath actuating parallel bars to the ends of which the jaws are attached, and are guided by the screws which hold

them, moving in the slots in the plate. The jaws are opened by pressing upon the other end of either of the bars with the thumb beneath the plate, while the forefinger of the same hand holds the plate above. Two spring clips are provided for refinishing old slides which have been mounted without centering. This turn-table is made by Beck of London and sold in this country by Wm. Walmsley and Co., of Philadelphia.]

[The Bausch and Lomb Optical Company make a turn-table which is provided with a hand rest, which when in use lies down in such a way as to project over the table and the slide, but not touching either, giving the hand a perfectly steady support in the manipulation of applying a ring of cement to the slide.]

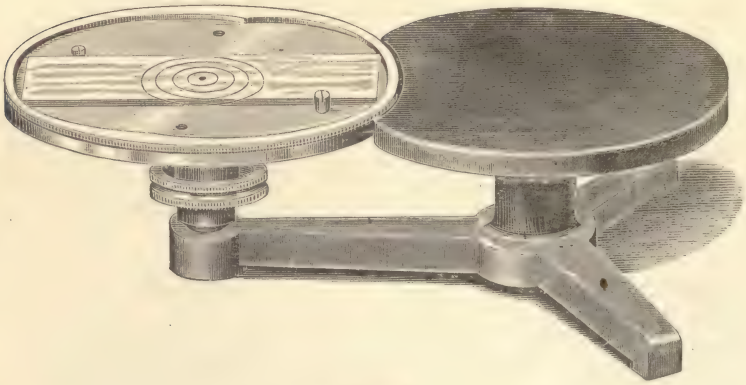


FIG. 106.

[Fig. 106 represents another form of the self-centering turn-table, made by Mr. Zentmayer of Philadelphia. The plan for centering the slide is something quite new. The slide is centered laterally by having its opposite sides brought in contact with two pins projecting from the plate. It is centered longitudinally by means of a ring with an oval inner edge, which is fitted to the periphery of the disk in such a way that by turning it, this inner edge of the ring grasps the slide at its diagonally opposite corners.]

[A form of apparatus for holding and centering the slide, and the mechanism for actuating it, which it is believed has certain

marked advantages in manipulation over other forms of self-centering turn-tables, has been contrived by the writer and is represented in Fig. 107. The general construction of the rotating plate, pivot, frame, etc., is the same as in Fig. 105. Fig. 107, *A* and *B*, represents an outline of the plate and attached apparatus ⅔ the natural size. In *A* the plate is seen from above, *aa* are two pins fixed in the plate equidistant from the center, by which the slide is laterally centered, *bb* are two pins movable in the slots *cc*, and, having a diameter above greater than

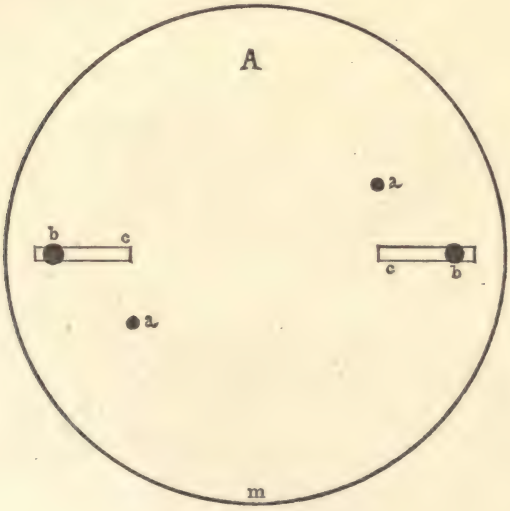


FIG. 107 *A*.

that of the slots, their shoulders bear upon the upper surface of the plate. These pins center the slide longitudinally. In *B* is shown the apparatus on the under side of the plate, by which the pins *bb* are moved in clasping, centering, and releasing the slide. These two pins *bb* are screwed fast to two short rods *oo*. The rods are joined to the long bent bars *tr tr* at *nn* with a hinge joint. These bars are bent at *uu* where they work upon pivots made fast to the plate. A pin 10 or 12 mm. long is inserted in the end of the outer bar at *x*. The short arms *rr* are

pressed outward at *x* by the strong steel spring *s*. This action brings the pins *bb* firmly against the middle of the ends of the slide, thus centering it longitudinally and holding it fast.]

[By grasping the plate at *m* between the thumb and forefinger of the left hand, the plate is held fast, while the finger presses against the pin at *x* and moves back the centering pins *bb* along the slots *cc*, while the right hand is left entirely free to manipulate the slide. An inward movement of *x* equal to 3 mm. will separate the pins *bb* a distance of 12 mm. The pin *e* pre-

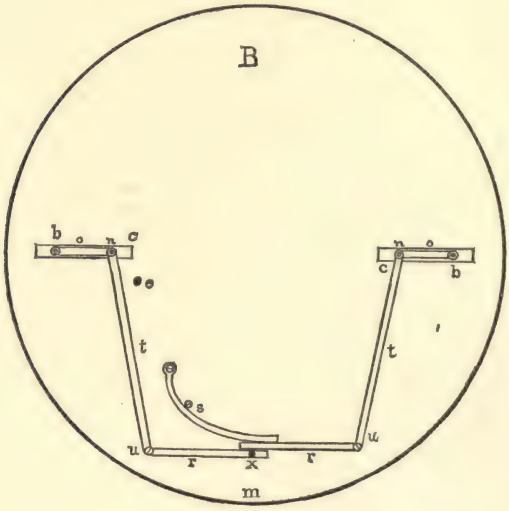


FIG. 107 *B*.

vents the centering pins *bb* from coming down to the ends of the slots at *cc* when no slide is on the plate. As it is screwed into the plate, it may be removed if very short slides are being used.]

[In the use of the self-centering turn-table the object itself must be placed in the center of the slide. When a cell is used and that cell has already been made by means of the self-centering turn-table, the object can be mounted and the cover adjusted by that. But when no cell or ring of cement has been

previously put on, which may serve as a guide, there must be something to indicate the central point of the slide. Two things may be done. We may place the slide on the turn-table in the usual way, and as it lies there self-centered, the concentric rings cut in the disk will be a sufficient guide for mounting the object and adjusting the cover-glass. Or we may lay the slide its poorest side up if there is any choice, on the turn-table, and with a hair pencil dipped in India ink draw a circle on it as near as possible to the size of the cover-glass to be used. When this is dry it will serve as a guide in placing the object and adjusting the cover, on the other side of the slide, near enough for all practical uses. When the slide goes on the turn-table for cementing if it is found that the cover-glass is not exactly concentric with the motion of the table it should be carefully and gently pushed over to its true place with a dissecting needle. It may not perhaps need to be moved half a millimeter, but it should be perfectly centered if possible before any cement is applied. A. B. H.]

Supposing now we have a preparation on the slide mounted in glycerine and a round cover-glass laid on, the glycerine having been so carefully measured out that no trace of it can be seen beyond the edge of the cover; on three or four points at the edge of the cover-glass is placed a drop of thick cement for a stay, as already described in regard to the rectangular glasses. When these are dry the slide is put on the turn-table and clamped and the cover-glass centered on the table. [Our author is not speaking here of the new self-centering turn-tables. With those described above it is obvious that the cover-glass must be made concentric with the turn-table before it is made fast with these temporary stays of cement. And, indeed, if glycerine is the mounting fluid, there will be no need of these at all if Canada balsam or the mounting fluid of gum mastic and dammar, described on p. 223, be used as the first layer to enclose the preparation. A. B. H.]

We can now proceed to apply the first ring of cement. Fill a small hair pencil with not too much of the cement, which we have chosen to use (there should be no drops of it hanging

from the pencil), hold it in a perpendicular direction close over the edge of the cover-glass and set the turn-table in slow motion. Suddenly, but gently, lower the pencil till it touches and the next moment raise it again. The ring is done. On the steadiness and accuracy of this motion alone depends the success of the process, and it can easily be attained. One should be careful not to take too much cement in his brush else the ring will not be uniform, particularly in breadth. A second and third ring, each broader than the preceding, should be laid upon the first. If a cement ring was laid upon the slide before the object was mounted and the cover-glass pressed down upon it, we shall not need the stay drops of cement, the glycerine will not be likely to run out, and the final cementing becomes a simple matter. The breadth of the last ring need not be more than 4 mm. The author has prepared many slides, which have remained unchanged for years, whose last cement ring with a diameter of 18 mm. was not more than 2 mm. broad. [The ring may be made as narrow as one pleases, by turning in the edge of it from the outside with the point of a knife as described in the paragraph on making cement cells, p. 232.]

[I am indebted to Rev. J. D. King for a process of sealing cells with heat that will be found very useful. The cell is made of shellac cement or lacquer finish and completed as already described. Before using, ring the outer half of the flattened top of the cell slightly with shellac cement. When the object is immersed in the glycerine or any aqueous mounting fluid, put on the cover, adjust it and press it down carefully to its bearings all around. Then apply a spring clip which has a gentle pressure and pass the slide, cover down, a few times over the flame of a spirit-lamp till the cement shows signs of melting. Remove the clip, press down the cover again at any point necessary and then hold it under the cold-water faucet to wash off the glycerine and cool it, after which carefully clean, and complete the cementing to fancy with shellac cement and lacquer finish. A. B. H.]

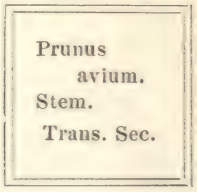
X. LABELING AND CATALOGUING THE PREPARATIONS.

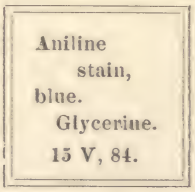
A. The Label. Every preparation must be carefully labeled in order to be easily found and identified. The proper labels have a rectangular form about as shown in Fig. 100, I, II, IV, or Fig. 98, or as it suits the taste of the preparator to make them. They are fastened on with a thick solution of gum arabic, or better still with a solution of brown shellac with absolute alcohol. With the latter, one may cover the whole upper surface of the label and so render the writing indestructible. In beginning a collection of microscopical preparations one should choose different colors for the labels and use each color for a limited group of objects. For example: white for anatomy of the vegetation organs of the phanerogams; blue, anatomy of flowers; green, vascular cryptogams; red, algæ; etc., etc.

The writing on the labels, of which every slide should have two, should include the following points:

1. Name of plant from which the preparation is made. For example, *Prunus avium.*
2. The part of the plant used in the preparation (stem).
3. The kind of section (transverse, radial or tangential.)
4. The method of preparation (aniline staining).
5. The mounting fluid (glycerine).
6. The date of mounting (15 V, 84).

The points 1–3 should be entered on the left hand label and 4–6 on the right:





Besides these labels it is well to write on the under side of the slide with a diamond the catalogue number of the specimen in order to identify it in case the left hand label should get lost.

[*B. The Catalogue.* Every collection of microscopical preparations should be carefully catalogued. As between the use of a book or a card catalogue I am inclined to prefer the latter; it seems to allow a somewhat freer classification of the object, with opportunities for throwing out specimens that are no longer desired, together with the card that represents them, without defacing the catalogue, as it would with a book by erasures. The catalogue should furnish a perfect index to the collection, each slide being represented by a card.]

[The classification of the preparations in the cabinet should be made in accordance with the natural system, in the main or primary divisions; and, in accordance with the parts of the plant which they represent, for the secondary division. For example: the primary divisions should distinguish between the phanerogamic and cryptogamic plants, and of the former between the monocotyledons and the dicotyledons and again, perhaps, of the latter of these between woody and herbaceous plants, and between deciduous and coniferous woods, etc. Under these the secondary divisions should recognize, and put together, sections or other preparations, made severally from the roots, stems, leaves, flowers and seeds of the plant.]

[The number of the slide then, after this arrangement, should represent its place in the cabinet. A Roman capital A, B, C, should represent the compartment of the cabinet in which it belongs, the Roman numerals I, II, III, etc., the particular box, drawer or tray which contains it, and the Arabic numerals 1, 2, 3, etc., the number of the slide in this particular holder. Thus each slide would be numbered in this way, B, XV, 15. The position of the cards in their box should answer exactly to that of the corresponding slides in the cabinet.]

[If an alphabetical index is desired in order to get the readiest possible access to any slide representing any plant in the collection, it may be had in a supplementary card index catalogue. arranged alphabetically according to genera and species. Let cards be printed with a blank for the proper scientific name of the plant from which the preparation is made, and ruled columns for the five minor heads, under which the slides are all classified, as follows:]

SCIENTIFIC NAME OF THE PLANT.				
ROOTS.	STEM.	LEAVES.	FLOWERS.	SEEDS.
B. XII. 9		B. XX. 6		
	B. XV. 23		B. XXI. 21.	
			B. XXI. 18.	B. XXV. 6.

[Thus a single index card could easily be made to represent at least 50 slides if necessary.]

[What should the catalogue cards contain? Various answers have been made to this. I will indicate what seems to me mos t important, following mainly the plan proposed by Prof. S. H. Gage* for catalogues of preparations of animal histology.]

[1. The scientific name of the plant.

2. The cabinet number of the preparation.

3. The part of the plant from which the preparation is made.

4. The special purpose of the preparation. What it is meant to show.

5. The special method of preparation. Whether it was previously hardened, softened, or cut in a natural state. Whether mounted whole, teased out into its elementary cells or fibers, or cut into sections, and if cut how, free-hand, or by microtome.

6. The bleaching or clarifying agent, if any, and how long a treatment was required.

7. The staining medium and time required.

8. The mounting, cementing and finishing media.

9. Objectives to be used in its study.

10. The date of preparation and name of preparator.

11. General remarks including references to literature and good figures. A. B. H.]

XI. STORING PERMANENT PREPARATIONS.

Finished preparations should be kept in a box or case, which is so arranged that the slides resting near each other occupy

* Prof. S. H. Gage, Proceed. Am. Soc. Microscopists, Chicago Meeting, 1883, p. 169, *ff.*

the least possible space. Microscopical cabinets should satisfy the following requirements. By shutting tightly they should protect the preparations from dust. They should not allow the slides to move about, and should permit them to lie in an horizontal position, where they may be most easily got at.

[For transporting slides special boxes should be used and not those in which they are usually contained. The principal optical firms in this country offer an assortment of object cabinets, which for convenience of arrangement and excellence of workmanship leave nothing to be desired. But they are mostly so costly as to be of the nature of a luxury, and most microscop-

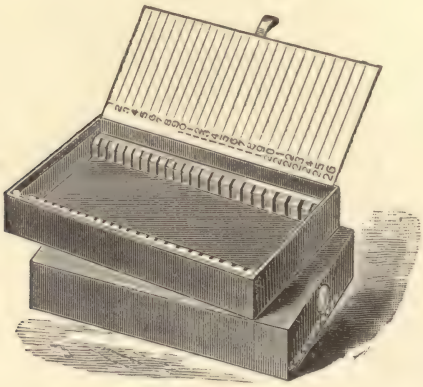


FIG. 108.

ists are obliged to satisfy themselves with the cheaper forms, such as wood or paper boxes with wooden racks, or construct a cabinet for themselves by some contrivance of drawers or trays which has the merit at least of cheapness if not of elegance.]

[Recently two adaptations of an old form of object box have been made which make them answer practical ends in a very satisfactory way and at a cost that brings them within the reach of all.]

[One, represented in Fig. 108 is a 26 slide box made of stiff paper board with wooden racks, and a hinged and indexed cover which when shut down holds the slides securely in place, and the whole shoves into a paper-board, cloth-bound case, shown

beneath the box in the illustration, which makes all secure. A circular blank is shown at the top wherein to write the number of the box for cataloguing. When the box is placed on end the slides are horizontal. These boxes may be kept on shelves like bound books.]

[The other is called "Pillsbury's Cabinet," and is shown in Fig. 109. It consists of a polished cherry cabinet containing twenty wooden-racked slide boxes, each holding twenty-five

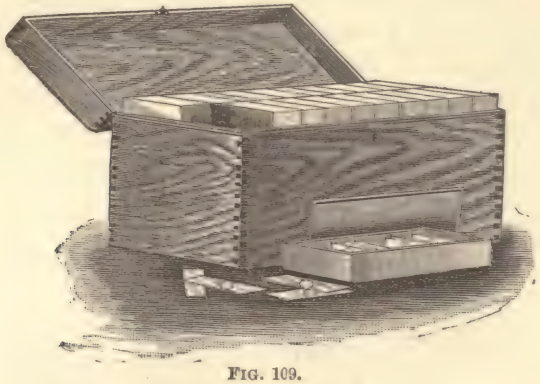


FIG. 109.

slides. In the illustration one box is shown removed from the case, with its cover off and some slides in place. The top end of each box, as placed in the cabinet, is provided with an index and on the bottom of the box inserted under each slide is a corresponding number. When the boxes are in place the slides lie horizontal and a list of all the slides which they contain is spread out before the eye. A cabinet of this kind capable of storing 500 slides is offered for $3.50. J. W. Queen and Co., Phila., make these two forms of slide holder. A. B. H.]

XII. THE EXAMINATION OF LIVING ORGANISMS.

There are numerous objects which may be examined under the microscope in a living state, as, for example, microscopic algæ and fungi. Some of these we might desire to keep under the microscope for a long time in order to observe their devel-

opment or their method of propagation. For this purpose a simple slide and the cover-glass and the object between are not very suitable, for the water quickly evaporates from under the cover-glass so that it frequently has to be renewed; this gradually increases the percentage of mineral substances held in solution in the water, and the object is soon brought into quite abnormal conditions for its life processes. Many contrivances have been invented for retarding or preventing the evaporation of the water. The older microscopists used a contrivance which consisted of two brass rings one of which screwed upon the other. Into each was fitted a little glass plate, the lower one made somewhat concave. The drop of water with the object was put into this and the other glass screwed down tightly upon it. With the older instruments of Schieck this apparatus had a diameter of 28 mm. and a height of 9 mm.

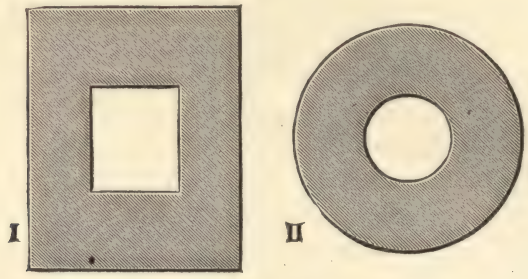


FIG. 110.

For a like purpose, a slide about 1.5 mm. thick with a small concavity about 13 mm. wide ground in it answers well. And laterally a thick slide (about 3.5 mm.) has been used in which a ring-like groove is cut about 3 mm. deep and of like breadth. [The "Weber life slide" sold by opticians in this country answers the same purpose even better. The bottom of the cell ground in this slide is convex. A. B. H.)

But by far the best method of observing living objects, or those under cultivation, is by means of the hanging or suspended drop. It may be arranged as follows.

A ring of wax is put on the slide making a pretty deep cell

and wide enough for the cover-glass used. On the cover-glass there is put a drop of water and the object in that. It is then turned quickly over so that the drop will hang suspended on the under side. The cover is then placed on the wax ring and put under the microscope. But the water soon dries up and the air also does not come to it freely. But all these objections are obviated by the use of the following little apparatus which is at once a ventilated moist-chamber and a hanging drop. It was Strasburger's[53] contrivance.

Cut from common tough pasteboard pieces like those represented in Fig. 110. The hole in the centre should be a little smaller than the cover-glass to be used. Then put the paper pieces in water and let them soak till they are thoroughly saturated. Take them out and lay two or three deep on a slide, and lay the cover-glass with its hanging drop over the central opening. The apparatus is now complete. I have kept a hanging drop unchanged upon such an apparatus for a fortnight by simply putting a few drops of water from the wash bottle upon the side of the paper every evening. By means of this moist-chamber I have been easily able in the spring of the year to observe[54] the *Spyrogyra* form its spores, while with other apparatus I have in most cases failed. With this apparatus too I have had much success in cultivating the pollen tubes in a 30 per cent solution of sugar, honey and like fluids.[55]

Geissler has constructed a very peculiarly formed moist-chamber for examining objects in a vacuum or with an atmosphere of carbonic acid or oxygen. It consists of a glass tube which is widened and flattened in the middle so as to form a smooth disk-shaped space, the upper and under walls of which are brought

[53] Strasburger, Befruchtung u. Zelltheilung. Jena, 1878, p. 5.

[54] See also Strasburger, *l. c.*, p. 5, *ff.*

[55] See also Strasburger, *l. c.*, p. 15–25, especially p. 16. Here it is said of the culture of the pollen of *Pinus pumilio*, "But by the prevalence of bacteria, of yeast cells and mould fungus, the culture will be ruined at farthest in 8 or 10 days. I kept it the longest when I used thyme oil in a thousand-fold dilution with 10 to 30 per cent sugar solution. This addition, however, at first hindered the formation of the tubes; but after about two days when a part of the thyme oil had evaporated they again began to develop (salicylic acid in a 1000 fold dilution kills the pollen grains), while the increase of the lower organizations which were introduced at the same time with the pollen grains was delayed for several days.

close together and are of the thickness of common cover-glass. The culture drop is brought between them. The tube allows a current of air to be thrown around it of any desired kind. This apparatus is especially applicable to the cultivation of fungus spores and the like.[53] A modification of the Geissler moist-chamber has recently been devised by Brefeld.[57]

[*The Gas Slide.* For the exposure, under the microscope, of organisms either animal or vegetable to the effect of certain reagents in the form of gases, for the sake of observing the effect of these gases upon the actions of living beings or upon the character of their dead tissues, it is only necessary to have a cell of glass, lying upon the stage, and supplied with tubes for the entrance and escape of the reagent. When constructed of glass cemented together, these instruments are liable to sep-

FIG. 111.

arate at the joints and are otherwise especially subject to accidental injury. The form shown in Fig. 111, is made almost wholly of brass, lies heavily and firmly upon the stage, and is safe from any considerable injury by breakage. The cover-glasses are easily accessible for cleaning, and if broken are easily replaced. Being metallic it transmits heat promptly, in case of use upon the hot stage. It it is made by T. H. McAllister of New York, at the suggestion of Dr. T. H. Hunt of Brooklyn. It consists of a heavy, slide-shaped brass box with a central, cylindrical perforation 20 mm. wide and 7 deep. This central well is closed at the bottom by a cover-glass cemented to the brass ledge on which it rests, and is covered, after the insertion and arrangement of the object, by another cover-glass which is

[56] Nägeli und Schwendener, Das Mikroskop, p. 275.

[57] Oscar Brefeld, Culture method for the investigation of fungi. (In Botanische Untersuchungen über Schimmelpilze. Untersuchungen aus dem Gesammtgebiete der Mykologie. Heft IV, 1881, p. 1–35).— See also Hansen's Chambre humide pour la culture des organismes microscopiques. Avec deux figures dans le texte [Meddelelser fra Carlsberg Laboratoriet, p. 181–183, Kjöbenhavn, 1881.]

held in place and rendered air-tight by a small quantity of paraffine, oil, glycerine, or other available material around the edge. Short brass tubes are provided, at the ends of the apparatus, to be attached to the tube bringing the gas to one side of the box and conveying it away after having passed through to the other. R. H. W.]

[*A Growing Slide*, or moist-chamber and hanging drop, shown in Fig. 112, is sold and used in this country. It consists of two common slides held together with rubber bands, the upper one perforated with a circular hole 10 or 12 mm. in diameter, over which the cover-glass with the culture drop is laid, being held by a little grease rubbed on about the edge of the hole. When the slide is not under observation it is laid in a flat dish containing a sufficient depth of water to overflow the lower slide and run in by capillary attraction between the two and so prevent the evaporation of the drop.]

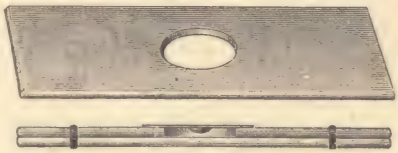


FIG. 112.

[*A Laboratory Table.* Prof. C. E. Bessey, professor of Botany and Horticulture in the University of Nebraska, kindly furnishes me with a plan of the tables used in the microscopical laboratory of that institution. It is represented in Fig. 113; *w* is the window, *a b c e* represents the form of the table, the breadth of which at *a b* is 1.5 m., and at *c e* .6 m., the perpendicular length 1.8 m. At the points indicated by *x* are placed the microscopes, each in such a position as to receive the unobstructed light from the window, without liability of interference from those working at the other microscopes. A. B. H.]

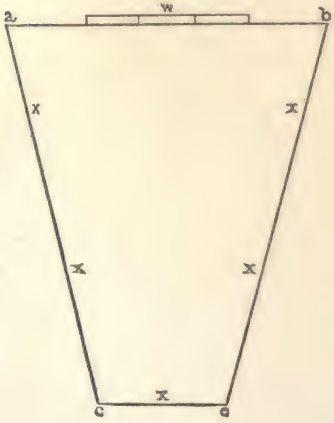


FIG. 113.

XIII. DRAWING MICROSCOPIC OBJECTS.

In the introduction of this work we have already shown how important it is to permanently fix the microscopic image by drawing. Farther along we became acquainted with some apparatus by which the image is reflected upon a piece of paper lying near the microscope so that the drawing could be done by simply tracing the outlines.

1. Aids to Microscopical Drawing.

An experienced draughtsman can draw free-hand the simple microscopic images by looking in the microscope now and then, drawing a part of the image which he has especially observed, comparing the drawing with the original to see if it fully corresponds, then taking up the next contiguous part and so on. This kind of drawing has this unqualified advantage, that in its use one is compelled to observe the image very exactly in respect to its forms and their respective relations. The practised draughtsman may by this method give to his drawing the greatest perfection. But this chapter is written less for the skilled artist than for those who are but little if at all proficient in the pictorial art. We shall therefore describe those methods which will assist the latter in making graphical representations.

To the unskilled it is a matter of the greatest difficulty to bring into proportion the large dimensions of the field of view with the smallness of the object, and, further, to rightly estimate the distance apart of the details of the object and to fix them. These difficulties are overcome by the following means.

(*a*) Small objects, which are considerably extended in one direction like diatoms and other algæ, may be drawn very easily with the help of the common ocular micrometer. This method enables us to draw the object in exactly the size in which it appears under a certain definite magnification. An example will immediately make this method clear.

I have a diatom, *Pinnularia viridis* Rabenh., to draw magnified 600 times, Fig. 114. I know that my micrometer scale

contains 4 millimetres each divided into 20 parts, and that in relation to my magnification of 600 this length is equal to 0.1 mm. (*i.e.*, 0.1 mm. in the object covers the whole scale in the ocular); the scale of the micrometer must also with the 600 fold magnification appear to be 60 mm. long.[58] I draw the scale in this length on a piece of paper, Fig. 114, dividing the whole scale into 8 equal parts, each = 7.5 mm., and these in halves. The long marks 0, 10, 20, etc., correspond to every ten divisions of the micrometer, the lines between standing for five divisions. I have already ascertained that the diatom measures 0.137 mm. which by 600 fold magnification must give a length of 82 mm.; by that I can easily fix the position of the points *e* and *f*, with the millimeter scale. I now bring the microscopic image, and

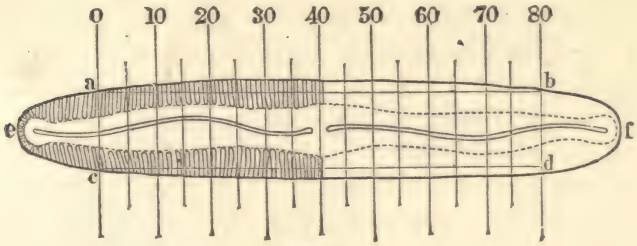


FIG. 114.

the scale in the ocular of the microscope into such a position as is shown in the illustration, Fig. 114. Now I can easily fix the outline of the diatom and the position of its separate parts on the drawing of my scale with the greatest exactness. Three markings of the *Pinnularia*, for example, correspond with one of the divisions of 5 on my scale. The two halves of the interrupted middle line are 3 divisions of the scale apart at the center. The various dimensions of the picture will be made to correspond to the reality since they are all laid out like a chart on a network of fixed lines. This kind of drawing is preferable to that with the camera lucida for diatoms of very delicate frustules since the delicate markings of these forms are seen with great difficulty in the reflected image.

[58] This value will naturally differ with every microscope and for each magnification.

(*b*) In the drawing of those images that extend uniformly over the field of view, the aid of the micrometer scale is not nearly as suitable. In this case drawing by free-hand is facilitated by the use of an ocular in whose diaphragm are cross threads which divide the field into four parts. One with double cross threads like the form shown in Fig. 115 is still better. Since this can seldom be bought I will explain how it can be made. Prepare a circular rim of brass about the size of Fig. 115, which may be fitted to the ocular tube from above. On this the cross threads may be made fast, which in low power oculars may be made of fine glass threads drawn out by means of the blow pipe flame, or of human hair previously boiled in alcohol to get the oil out. In high power oculars the cross threads should be made of spider's web. The preparation of the latter is by no means so difficult as is generally supposed, only one must not stretch them — and the same is true of hairs — when the air is dry, since afterwards the fiber by taking up moisture in a humid atmosphere would contract and break. First mark the places on the brass rim where the fibers are to go and then fasten one end on one side by means of wax and draw the thread over to the other side, fastening it in the same way, warming the wax to make it soft. Having made the fibers fast on the rim put some Canada balsam on the lower side of it and drop it carefully into the ocular down upon the diaphragm where it will stick fast. After the Canada balsam is dry screw on the eye lens and afterwards remove it as seldom as possible.

FIG. 115.

How then shall we apply this contrivance to the drawing of microscopical pictures? Fig. 116 represents a schlerenchyma bundle in a cross section of the root of *Pteris aquilina* which we will draw by means of the double cross threads magnified 600 diameters. This is easily done when we have first learned the size of the square by direct measurement.[59] In the other drawing with this magnification, the length of our micrometer

[59] Lay the crossed threads under the microscope as an object screw on a low power *n*, and draw it in its natural size with a *camera lucida*. The length of the side is then measured with the millimeter scale and divided by *n*; the quotient gives the exact length in millimeters.

scale, 4 mm. as it appeared in the ocular was equal to 0.1 mm. used as an object, and magnified 600 times. We have also ascertained by measurement that the length of one side of the middle square is 2.2 mm. Consequently under the same conditions this side will appear to be 33. mm. long, for:

$$4 : 0.1 \times 600 : : 2.2 : x$$

$$x = 33.$$

Now we remark that the upper side of the square *al*, Fig. 116, covers three cells. It will be easy to estimate their respective

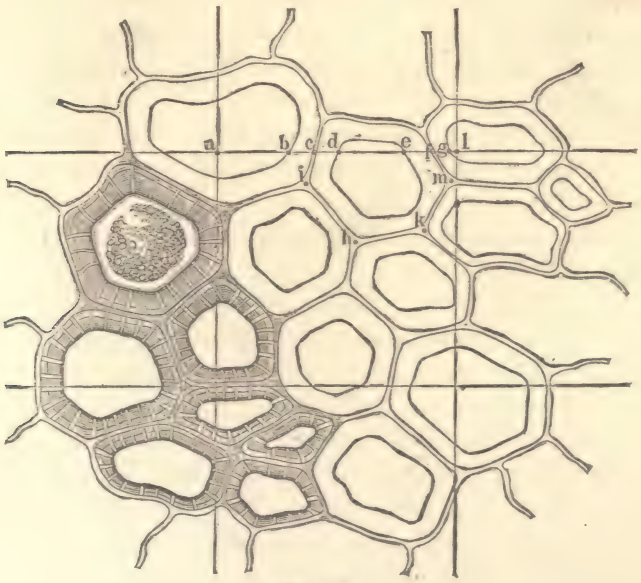


FIG. 116.

lengths and to fix the points *b*, *c*, *d*, *e*, *f*, *g*, also like points on the other three squares, and the uniting points of the cellular network which fall inside the squares *i*, *h*, *k*, *m*, are likewise determined without difficulty by comparative estimation. When they are all determined for a given cell then the outline will be drawn, and so on. In this way we are able, without very great errors, to map out a group of cells like these, and, what is of greater importance, we soon attain by this method a certain readiness and skill in estimating sizes and distances under the microscope.

This readiness will very often be of use to the microscopist. On the right side of the illustration, the network of cells is shown drawn in outline; the rest of the cells are more fully drawn.

(*c*) In most cases when a microscopic image is to be traced, recourse will be had to some camera lucida already described. The use of this very helpful apparatus is very soon and very easily learned. There is therefore need of saying but a few words about it.

The eye should be held close to the opening, provided for seeing in the instrument and look perpendicularly down, for by looking obliquely the image may be considerably distorted. The paper upon which the drawing is to be made should be fastened lying flat, at a standard distance of 25.4 cm. from the camera lucida. It is best to provide a drawing board on which the paper may be fastened and which may be placed at this distance from the camera.

In order to draw a picture by means of the camera lucida without painfully straining the eyes, it is necessary that the microscopic image, and the paper and pencil be uniformly illuminated. If the image has, in comparison with the paper, too strong a light the pencil will be seen with difficulty if at all. On the contrary, if the paper in comparison to the image be too strongly illuminated, the delicate outlines of the latter will be indistinct. The first usually occurs where the image of the paper and pencil is thrown into the field of view of the microscope and the latter when the microscopic image is reflected upon the paper. This difficulty may be remedied by throwing either the image or the paper into a shadow. Both may be done simply with the hand, or by a properly constructed screen of paper,* or by a disk of pasteboard set up at some distance, and the like. Hartnack provides his cameras with a blue glass plate, which partly obscures the light, and this appears in fact to have been applied to the construction of many cameras.[60] A few trials with the microscope with different magnifications will afford the necessary experience for properly managing the light.

* See Note, page 116, for description of a handy form.

[60] See C. Cramer in Botan. Centralbl. 1881, Bd. VII, pp. 387–391.

In tracing the outlines of the image under the camera, the pencil used should not be too hard and the lines should be very light, and then they will often appear rough, for the position which one has to take in this work is not very favorable to nice drawing.

2. Conducting Microscopical Drawing.

How a microscopical drawing should be carried out depends upon what relations, qualities and observations we wish pictorially to express. We have already remarked that a microscopical anatomical drawing should by no means be a mere copy of the image seen, but that it should reproduce the sum of the experiences which the observer has had with his preparation. Furthermore, in most cases the drawing should show only those relationships which the observer has arrived at by his analysis;

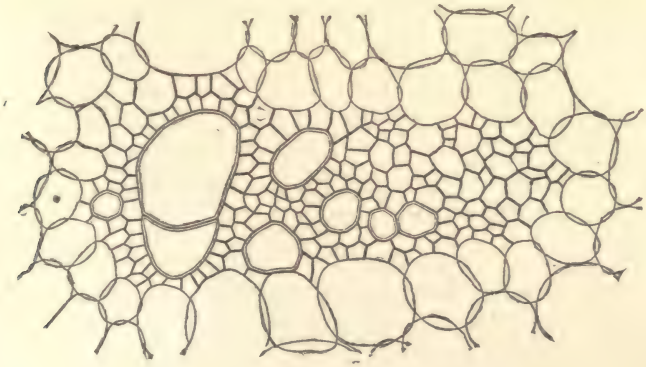


Fig. 117.

hence it will often not by far show all of those parts which are in reality seen in the microscopic image. Suppose, for example, that one makes a purely histological study in order to represent the *relative position* of the cells in the tissue; certainly he will not need to draw the contents of each single cell, its protoplasm, nucleus, etc., or the finer markings of the cell-wall which may temporarily interest him. Take a concrete example. Some one studies the anatomy of the stem of *Richardia africana*. He wishes to know the vascular bundles in respect to their form,

their position and their structure. In this case it will be necessary to draw only the outlines of the cells, and their respective attachments and perhaps also the relative thickness of their walls. His drawing, Fig. 117, will therefore represent the cell walls with single lines not doubly outlined as they are in fact. He will, perhaps, in order to make it perfectly understandable to another, express the cell walls of the surrounding parenchyma tissue by the more delicate lines, and the cambiform, by the stronger lines, and the vascular walls by the strongest, or by double outlining. Such a drawing is naturally in the highest degree diagrammatic, but it perfectly satisfies all demands

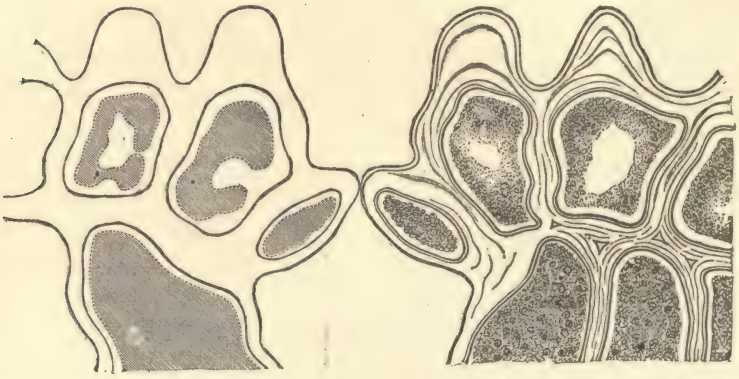


FIG. 118.

made upon it, and is to be preferred to any drawing which with a mere photographic fidelity reproduces the microscopic image, in that it does not divert the eye from the principal thing by the presence of unessential accessories.

Take another example by which we can see, in the same illustration, the distinction between a diagrammatic and a completed drawing. Fig. 118 represents a highly magnified trans-section through the upper part of the stomata, and the adjacent epidermis, of the needle of *Taxus baccata*. The left half of the drawing is diagrammatic, the other is completed. The left teaches us to know only the form and size of the cells, the substance of the cell wall and the casual parting of the protoplasmic cell contents. The right half shows us all these relations, and further

the extension and form of the cuticle, the structure of the cell wall, the intercellular substance, and the different appearances of the cell contents in the stomata-closing cells, the epidermis and the sub-epidermal layer. The right half shows us *all* the relations which we are able to deduce from the portion of the preparation examined and with the magnification employed.

It is self-evident that one may make a microscopical drawing more or less diagrammatic according to the requirements of each case. Fig. 119 represents the wood cells of a young coniferous plant. I is the most, III the least, diagrammatic. In I the middle

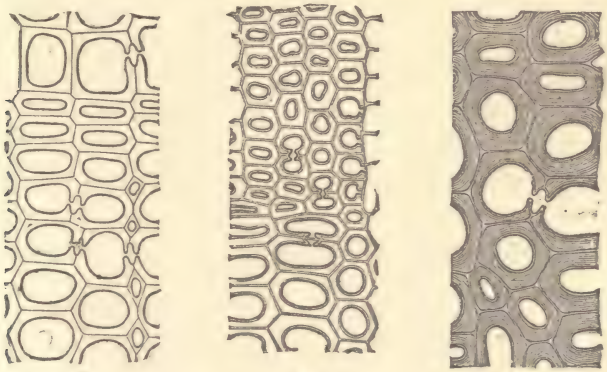


FIG. 119.

lamella is represented by a simple thin line, while the inward thickening layer is indicated by a heavy single line; the wood layer between is not designated further. In II the middle lamella is indicated by two delicate lines, the inner layer by a delicate and a heavy line, the latter being the boundary line of the cell cavity while the woody layer is not further exhibited. In III at last the concentric layers of the latter are represented. A quite perfect picture of the cell wall structure is shown in this drawing, as one sees it with a high magnification.

The representation of cell walls in microscopical drawing is by no means difficult since all that is required is uniform concentric outlines. The difficulty of representation increases when fluid, or semifluid cell contents are to be pictured. If the cell contents are a clear, homogeneous fluid, we must depart from a

strictly pictorial representation since a fluid shows itself under the microscope only by its refraction of light. At most it can be diagrammatically represented only by shading or laying in with the rubber. But it is otherwise with the protoplasm and its granules and air bubbles, which may be very well and very beautifully reproduced by drawing. It may be done either with the drawing pen and India ink or with the lead pencil. Suppose we are to draw the protoplasmic contents of one of the upper, right hand cells of Fig. 118. We first make the outlines of the granular substance with fine points. Then we fill in the space pretty uniformly with very delicate points. Then in those places which in the microscope appear to be more densely granulated we add a corresponding quantity of points, so that they stand thicker and here and there touch each other. And finally the larger and largest grains are represented by minute circles or irregular outlines according to the appearance of the object. If the protoplasm is very thick and cloudy it will not do to make the background of delicate little points, but it should be made of many finely entangled lines running through and through each other as is seen in the lower cells on the right hand side of Fig. 118.

In the examples thus far treated we have dealt with the reproduction of images which are seen with a single adjustment of the microscope, but we shall often be required to combine several adjustments, and so make a drawing which at least in places represents corporeal thickness. As is well known, this can be accomplished only by the addition of shading, and nowhere will there be more faults committed than right here, as Nägeli has well said. In botanical literature there are many drawings of this kind, in which the body is represented as at the same time transparent and untransparent, as flat and wavy, and as round and angular. In order to draw, at least approximately right, we must think always of a definite direction from which the light falls upon the body to be shaded, for example, from the right or left above at an angle of 45°.

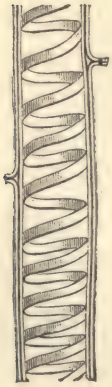


FIG. 120.

Very often partly corporeal representations are of spiral tissue such as is pictured in Fig. 120. We immediately see from the drawing that the wall of the tube is not here corporeally or perspectively represented, but only the inclosed spiral band. The walls are given as they would be seen by one medium adjustment of the lens, but the spiral band represents several adjustments combined into one view. It supposes the light to enter from the upper right hand side.

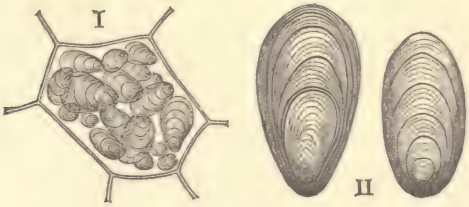


FIG. 121.

Shading is particularly required where it is desired to represent any cell-contents which have a peculiar form. Included in this are chiefly grains of starch, chlorophyll and coloring matter, likewise crystals and drops of fluid and air-bubbles in fluids. Little need be said concerning the representation of starch grains since they occur in many forms simple and compound. As an illustration we give a picture of the well-known starch grain of the potato, Fig. 121; the illustration shows two single grains of starch which are shaded as if the light fell from above in an oblique direction. It is impossible by this kind of shading to make the impression that the grain is a flat disk, as by a want of shadow it might easily appear to be. Chlorophyll grains must be represented commonly as rough little balls, as shown in Fig. 122, I. If we wish to show highly magnified chlorophyll grains with starch grains on the inside,

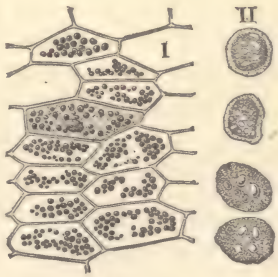


FIG. 122.

we have recourse to the forms indicated in the lower granules of Fig. 122, II. Small solid granules of another kind occur more rarely, for example in the yellow-colored floral leaves. If very small they are represented by simple circles, as in Fig. 123, which is a longitudinal section of the head of the nectar hair of *Parnassia palustris*. If they are larger they may have the proper shading of a globe as indicated in the sketch, Fig. 123. Crystals which occur in plant cells, must always be drawn solid, in order to make the right impression upon the beholder; they may be shown in outline, or, what is better, shaded. Of the manifold forms of microscopic crystals, we give in Fig. 124 the three which occur most frequently, in order to exhibit the kind of shading to be used. It becomes clear from the shading that the middle figure in the illustration is a quadra-octahedral crystal with truncated point; the one on the right, on the contrary, is a rhomboid.

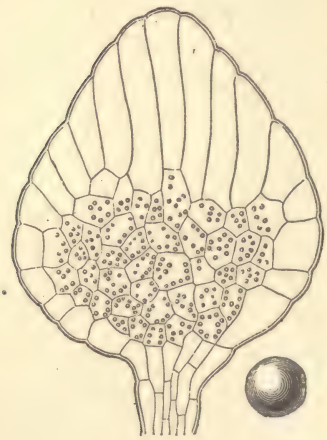


FIG. 123.

The representation of small drops of fluid or of air bubbles is attended with more difficulty since their appearance changes with the adjustment of the objective. Focussed in the middle an air bubble appears sharp and with a double outline, while if the focussing is higher up it assumes the form of a dark colored globe which becomes brighter toward the poles, and at the pole itself brightly sparkles. The pictorial representation is commonly by two concentric circles which stand apart about as the outlines are perceived to do with the medium adjustment of the lens.

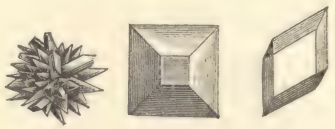


FIG. 124.

It need scarcely be added that the foregoing analysis includes only a few of the more frequently occurring cases. On the

other hand, we must pass over to the practical draughtsman a great many things, to find out for himself the best way of drawing them. He will be much helped by carefully studying good drawings of objects which are like his preparations. Of works which contain the best illustrations for this purpose we commend Sachs' Text Book of Botany; DeBary, in Sachs', Hofmeister's, and DeBary's Manuals; Lürssen's Medico-pharmaceutical Botany; Strasburger's "Cell-formation and Cell-division"; Kny's Botanical wall charts; likewise numerous illustrated monographs in Pringsheim's Year Book and in the "Botanischen Zeitung."

3. Drawing Materials.

Most anatomical drawing will be done with the lead pencil which should not be too hard if one wants delicate soft pictures. Faber's Nos. 2 and 3, are best and especially the yellow hexagonal forms. The pencil should have a very fine point and this can be best made by the use of a file. Shadings, cloudy parts, delicate cell-contents can be most conveniently put in by means of a soft leather rubber, which gives to the drawing a very neat and at the same time natural appearance.

For drawings that are to serve as guides in lithographing it is better to use the drawing-pen and India-ink, rather than the pencil, thus making the so-called "stipple drawing" so like the lithograph that one may be reasonably assured that no strange additions will be made to it in lithographing it. India-ink drawings also serve well for photographic transfers, but for this purpose cinnabar color should be mixed with the India-ink.

For coloring microscopical drawings either oil-crayons or water colors are most to be commended. In the use of the oil-crayons the paper does not need to be previously touched up with the rubber eraser. Sussuers' oil-crayons allow different colors to be drawn over each other so that one can obtain with them any desired shade. In respect to water colors, the fluid colors in tubes are to be preferred before all others. In recent times they have been extensively applied to other purposes.

For putting on colors use the sable pencil, or the common India-ink pencil with a long handle.

Of the kind of paper to be used choose a good number of "Watman" which is not too rough. For India-ink drawing a well smoothed cardboard is best. For preliminary sketches make a good quality of writing paper do, as the Watman paper is too expensive.

CHAPTER IV.

MICROSCOPICAL REAGENTS.

I. Introduction.

If we make a section through some plant tissue, for example, a cross section through a young wood stem, and examine it under the microscope in water, glycerine, or other uncolored fluid it will — with the exception of a few unimportant cases — appear throughout uncolored. This colorlessness will show itself not only in the membranes and walls of most cells, but also in most of the fluid and solid contents of the cells. It is obvious that it would frequently be very difficult to determine, at sight, as to the nature of the small bubbles, drops and granules in the interior of the cells. It is quite otherwise with, for example, the widely distributed chlorophyll which by reason of its green color is very easily recognized, even by the beginner. If, on the other hand, the small drops of oil and fat, the granular substances containing proteids, etc., were provided with a color peculiar to themselves, they would be much more easily identified than they now are.

Quite early, indeed in the first years of our century, it was observed that if a fluid containing free iodine in solution were added to cells containing starch grains, the grains became blue under the influence of the iodine while the other parts of the cell-body remained uncolored, or were at least of another color than blue. Nägeli afterwards showed that the blue coloring was produced by the particles of iodine entering the starch grain with a characteristic molecular intercalation.

Soon after that Theodor Hartig found that an ammoniacal solution of carmine behaved in a very remarkable way towards the protoplasmic contents of the cell, also to the nucleus and to the protoplasm in the restricted sense of the term. While these

substances in a living state behaved themselves quite indifferently towards the red colored fluid, the nucleus immediately after death eagerly absorbed the carmine substance, and in consequence was stained a beautiful carmine red. While previously the nucleus of many cells could be recognized with the greatest difficulty, they were now observed without trouble. The dead protoplasm at first continued perfectly colorless under the influence of the carmine fluid, but after a long time it absorbed the carmine color, yet with a much more delicate shade than the nucleus.

It has long been known to the chemist that ferrous salts which contain but very small traces of ferric oxide, enter into complete chemical combinations with tannic acid, which are partly distinguished in aqueous solutions in the form of dark blue or dark green precipitates. So, when cells containing tannic acid are impregnated with this iron compound, a precipitate is immediately produced which has this color and demonstrates the presence of tannic acid.

From these few examples it follows that there are certain substances available to the microscopist by which he is able to give account of the nature of the compound materials of plants. These substances are called *microscopical reagents*, and the effect which they produce upon the parts of the plant is designated the *reaction*, and the methodical application of the reagents, in order to recognize the nature of the framework or the contents of the cell, *microscopical analysis*.

The effects of microscopical reagents are of various kinds. If the reagent unites with the material to be investigated forming new combinations, the effect in this case is *chemical*. The reaction described just now on tannic acid is founded on a chemical effect. If proteid substances are treated with nitric acid and ammonia there is produced with a brown coloring a xanthoproteid salt of the alkali. This is another example of the effect of a chemical reagent.

A great many microscopical reagents, however, produce effects upon the substances under investigation very different from those described above; namely, physical effects. For example, we lay a transverse section of the young stem of *Lonicera* in a mixture of aniline red (fuchsin) and aniline blue dissolved

in alcohol for a short time, then pass it through absolute alcohol and wash it out in distilled water and put it under the microscope for examination in water or glycerine, it will show that the different layers of fibers have absorbed in part different colors. The walls of the outer layer of bark have become slightly bluish, while the inner bark layer remains almost colorless. The bast vessels and a part of the soft bast appear reddish. The cambium zone is perfectly uncolored. The vascular bundles are a beautiful violet, the wood parenchyma only being uncolored. The outer layers of pith are dark blue, the inner quite uncolored. These different colorings rest upon the fact that the red and blue stains have mechanically penetrated those cell walls which have a certain structure. There has taken place a molecular intercalation in the tissue of the walls but no chemical combination with the substance of them. The uncolored parts possess a certain physical quality which does not admit either of the pigments to penetrate them, the red colored absorb the fuchsin, the blue only the aniline blue, while the violet takes up both. It can be easily shown that no chemical change has taken place in the cell wall by reason of the action of the coloring substances. If the section be laid for a considerable time in absolute alcohol the colors entirely disappear. They have been leached out by the alcohol. Now the section will submit to any other reagents giving precisely the same reactions as though it had not been colored at all.

Since a large number of microscopical reagents exercise a purely physical effect it is essentially false to speak of a *microchemical analysis* or to designate the doctrine of microscopical reagents and their application with the expression "micro-chemistry."

Whether a microscopical reagent produces a chemical or physical effect is a matter of no distinct consequence, and microscopical analysis attributes to both categories of reagents the same value. The problem is first to find ways and means for establishing the chemical constitution of the substances of which plants are made, and second to make the delicate structural relations, in the investigation of which we come upon our chief difficulties, stand out more sharply. It accomplishes this mainly

in one way. It is naturally self-evident that the microscope must, in every single case (if it be not otherwise possible), make it clear what the effect of the reagent which we employ really is, though it need not be concealed that we still know very little of the way and manner of the working of many reagents.

Microscopical reagents and their effects constitute a purely empirical science. We are indebted to experiments for the possession of most of the reagents. Those discovered by scientific deduction are the small minority. It is not to be denied that a great many proposed reagents are of no very great importance, and give but doubtful results. The effect of others which have been much commended by their discoverers has not yet been sufficiently verified to allow them without further trial to be set down as good reagents. Microscopical analysis generally is of too recent a date to be considered in any respect concluded; on the contrary, its wider cultivation belongs to the future.

The older phytotomists indeed employed single reagents now and then, but it was done only incidentally and the results were considered of no great importance. The first who emphasized the eminent significance of a methodical application of microscopical reagents was Theodor Hartig. By their aid he came to know many anatomical relations which escaped the notice of his contemporaries and which were generally rejected by them, for Harting constructed a nomenclature of his own which differed much from that of other botanists, and he gruffly rejected all views opposed to his own. Not until recent times has it come to be recognized more and more that Hartig was far in advance of his contemporaries in the interpretation of many anatomical structural relations. He came to a right knowledge of these in many cases by the use of microscopical reagents. The number of botanists who afterwards essentially contributed to the cultivation of microscopical analysis is quite large. We shall become acquainted with their individual contributions in the course of this and the following chapters. Here, first of all, may be given those names which take the most conspicuous places in the history of microscopical analysis. Nägeli is first to be mentioned, who by his classical investigations of the in-

fluence of iodine reagents upon starch, cellulose, etc., produced a model work; then came Sachs who found a series of new and beautiful reactions and methodically applied the same in extraordinary investigations of the germination of seeds; next was Hanstein to whom we are indebted likewise for a number of important reactions. Finally, we name Strasburger and Wiesner whose numerous discoveries in this line will be enumerated further on.

Statements concerning the methods of microscopical reactions are found widely distributed in anatomical and physiological literature. With the exception of an early list of reagents by Theodor Hartig[1] a serviceable catalogue of them has never been attempted. There are to be sure in the works of Nägeli and Schwendener, Dippel, Frey and others, so often cited above, many incidental statements upon this subject, but they are either very short or very hard to find, or are no longer accepted. While the preparation of these chapters was going on, there appeared a very careful brief compendium of the facts belonging to this subject by V. A. Poulsen under the title "Botanisk Mikokemi, Vejledning ved fytohistologiske Undersogelser til Brug for studerende."[2] We can commend this little work in the warmest terms for the instruction of all beginners in that department.

In the remaining part of this treatise we propose to furnish a complete survey of microscopical reagents, their preparation, application, and the effect which they produce upon the organs of the plant. In the great quantity of data which had been wrought out it was not possible to name all the known facts, not indeed because it would thereby unreasonably increase the size of the work. There is a whole series of reactions whose usefulness is by no means securely established which must be excluded for this reason. Attention can be drawn to them only by brief references to the literature which treats of them. In the main, the author has striven to give all references to the related literature with the utmost completeness, in order to spare

[1] Hartig, Entwicklungsgeschichte des Pflanzenkeims. Leipzig, 1858, pp. 153–156.

[2] Kopenhagen, 1880.—Also appeared in a German translation by C. Müller, 1881. Also an English translation of the German edition, 1883, published by S. E. Cassino and Co., Boston, Mass.

the reader time-consuming and often fruitless searching. Whenever it was possible the reactions were carefully tested, as will be seen by a number of records relating thereto.

II. APPARATUS FOR THE PREPARATION OF REAGENTS.

In almost all cases the microscopist must prepare his own reagents. Particularly is this necessary if he desires them to be absolutely pure. The reagents are simple fluids, or mixtures of several, or finally solutions of solid substances in simple fluids or fluid mixtures.

The prepared reagents should be kept in glass vessels. For many, a common medicine bottle closed with a cork will be

FIG. 125.

FIG. 126.

perfectly suitable, though it would be better to use a glass-stoppered bottle whose stopple is ground to fit air-tight. The handiest are those whose stopple is prolonged into a long point which dips into the fluid and is very convenient in taking out the required drop of the reagent, Fig. 125. For all such reagents as at the common temperature produce vapors which would be likely to harm the glass lenses of the microscope, or the worker

himself, a glass-stoppered vessel with a double inclosure should be employed, Fig. 126. It has a glass stopple with slender prolongation well ground in. The upper part of the vessel is suddenly drawn in and a glass bell is fitted on over the contraction. The under edge of the bell and the contracted part of the bottle are ground to fit each other so that the bell hermetically closes the upper part of the flask outwardly. These bottles are especially recommended for ammonia, ether, muriatic, nitric, sulphuric and acetic acids.

The necessary manipulations in the preparation of the reagents are, weighing solid substances and fluids, measuring fluids, pulverizing solid substances, and heating, distilling and filtering fluids. Pulverizing solids should be done in a porcelain mortar. For heating or distilling use, according to the nature and quantity of the fluid employed, a test tube, a glass alembic, a small retort, watch-glass or porcelain saucer, which may be set during the process on the wire netting stretched over the tripod, or upon a retort holder. As a source of heat use a spirit lamp or a Bunsen gas burner. The operation of filtering deserves special care and attention because if one use an impure filter, he is likely to transfer a small quantity of foreign matter to his reagent, which possibly may disastrously interfere with the reaction. The filter should be prepared by putting a considerable number of good filter papers in a glass cup and pouring upon them pure dilute hydrochloric acid, letting them remain in the same from thirty to sixty minutes; then wash with distilled water, as long as litmus paper shows any acid reaction and dry with moderate heat. By the use of these purified filters we are sufficiently insured against contaminating the reagent in the act of filtering. Many substances, like a solution of caustic potash, mineral acid, etc., cannot be filtered through paper but must be filtered through glass wool. For this purpose put a small quantity of the glass wool which has been carefully washed and dried in the bottom of a small funnel and pour on the fluid. Glass wool is much to be preferred to asbestos which was formerly used.[3]

It is often not very easy to determine if the substances used

[3] Asbestos wool will answer, however, when the glass cannot be had. A. B. H.

are in truth chemically pure, that is if they contain no admixture of foreign matter which might make them unsuitable for use as microscopical reagents. In this matter it is evident that qualitative analysis can give the only safe information, and reference must be made to the numerous works and guides which treat of this subject. Also the methods for purifying the substances to be used (excepting the cases to be described farther on) may be looked up in chemical works.

For the ordinary purposes of the microscopist an apothecary's scales are fully sufficient for determining the parts by weight of solid substances and of fluids. If it be well constructed it allows weighing from 1 to 2 mg., and this exactness is quite sufficient. In order to prevent contamination in the process of weighing, solid substances should never be weighed on the open scale of the balance but on a watch-glass which has been previously cleansed with care. The weight of the glass can be determined once for all and marked on the glass with a writing diamond.

The determination of the parts of space or volume of fluids is done with graduated glass flasks, the so-called measuring flasks. The forms of glasses especially commended for the use of the microscopist are the following:

The Measuring Flask. (Fig. 127.) This apparatus is commonly a glass retort with a long narrow neck, with a mark on its lower third. The vessel is filled up to this mark with a fluid which is then poured out into another vessel and measured to the last drop. The cubic centimeters which it measures are then etched upon the side of the flask and become its "signature." It is advised to have several of them holding respectively 100, 150, 200, 250, and 1000 cc. Smaller quantities of fluid are measured out by means of the well-known "pipette," two useful forms of which are given in Fig. 128. Draw up the fluid in them with the mouth till it stands above the mark. Then close the upper end of the pipette with

FIG. 127.

the damp index finger and carefully let as much of the fluid run back as will bring it down exactly to the mark, after which empty the measured contents into the vessel intended for it. Finally, put the end of the pipette from which the fluid flows against the side of the vessel, but without blowing in it, and the vessel will contain exactly the quantity of fluid indicated in the signature of the pipette. Pipettes of 5, 10, and 25 cc. capacity are sufficient.

Still smaller quantities of fluid may be measured by the measuring cylinder, a small form of which is given in Fig. 129. Suppose we were to measure out 1.5 cc. of water. Fill up the vessel near to the desired quantity. Let a part run out till the surface stands at some one of the dividing lines, and now pour slowly, drop by drop at last, till the fluid exactly reaches the mark 1.5 cc. on the cylinder when it is held to the light perpendicularly before the eye. One may employ the siphon illustrated in Fig. 75, p. 162, for taking up small quantities of fluid. It should, however, be previously moistened within and without with the fluid to be used.

Fig. 128.

Fig. 129.

For many purposes requiring very exact measuring the so-called "Burette" is indispensable. The two most serviceable forms are the "bulb-burette," made on the principle of Guy-Lussac and modified by Mohr, and Mohr's "spring compressor burette." Both forms are illustrated in Fig. 130.

The Bulb Burette consists of a somewhat thick walled glass tube, *b*, about 400 mm. long which is rounded at the lower end and fitted into a wooden foot, *a*, so as to stand perpendicularly. Its upper end is closed with a cork in which are bored two holes

into which are fitted two glass tubes. The one, *c*, angularly bent, extends its long limb almost to the bottom of the burette. The tube, *d*, is less acutely bent and extends but a little way below the cork, while upon the upper end is fixed a rubber ball having an opening on the side which can be closed with the finger. When the burette is filled with fluid, a certain quantity of it may be forced out through *c*, by pressing on the bulb while the thumb closes the orifice. The rapidity of the outflow may be perfectly regulated by the pressure upon the bulb. The bur-

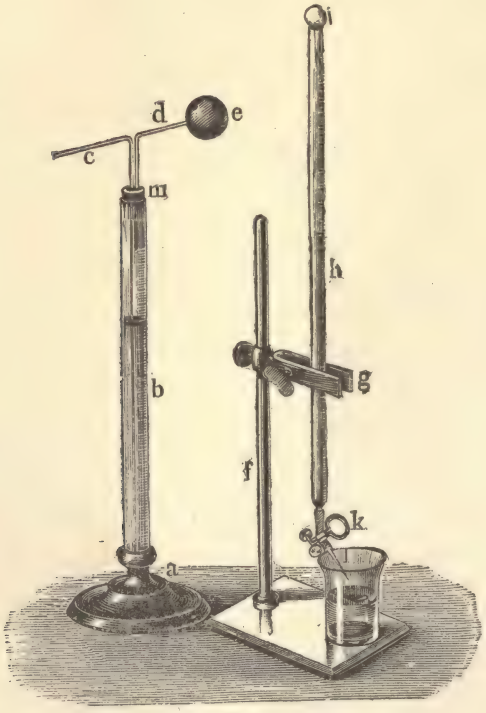


FIG. 130.

ette is filled by dipping the end of the tube, *c*, in the fluid and sucking at the opening in *e*. The tube, *b*, has the graduation in cubic centimeters (holding from 40 to 60) and fractions of the same to about 0.2. The value refers to the interior diameter of *b*, and the tube *c*, which dips into the fluid.

Mohr's Spring Compressor Burette consists of a long tube, *h*, divided into whole and fifths of a cc., open above at *i*, and below at *k*. The lower end is much narrowed and a piece of rubber tube about 27 mm. long is drawn over it. This is provided with a small glass tube having its point drawn out as at *l*. The rubber tube is closed by means of a spring compressor, *k*, and the burette is supported at *g*, on the stand, *f*, in a perpendicular position. The tube is filled by means of a small funnel and the opening at top is afterwards closed by a small glass globe, with a peg upon it, which excludes the dust. The outflow of the fluid to be measured is effected by partly opening the spring compressor.

Mohr's spring compressor burette is used chiefly with those indifferent fluids which would not affect the rubber tube, while those which would damage it should be put into the bulb burette where they will come in contact only with glass.[4]

The measuring vessels here described allow us, first, to measure any desired volume of fluid, but they can also, secondly, be applied to measure quantities of fluids by weight when they have been changed into equivalents in volume.

Take water for instance. It is known that 1 cc. of water, at 4° C., weighs 1 g. So if one has 30.5 cc. to weigh out he would get the right weight by measuring, at 4° C., 30.5 cc. of water. If he has to perform the operation at the common temperature of the room (say 17° C.) then the 30.5 cc. of water would weigh somewhat less than 30.5 g., since the water has a less degree of density at 17° than at 4°, but this difference is so inconsiderable that it may be disregarded in our work. According to the investigations of Despretz, if the volume of a gram of water at 4° C. be 1 cc. at 17° C. it will be 1.00120 cc. Hence the volume of 30.5 g. at 17° C. would be 30.5366 cc. But the excess of 0.036 cc. is far less than the error one would make in reading off the scale.

[4] In regard to getting the caliber of measuring vessels, *cf.* Bunsen, gasometric method (Brunswick, 1857), pp. 26-36. Concerning testing and correcting the same, *cf.* F. Mohr, Lehrb. d. Chem-analyt., Volumetric Method (4 Aufl., Brswg., 1874). pp. 1-50. further, Fresenius Anleit. z. quantitative chem. analyse (6 Aufl., Brschwg., 1875), pp. 36-46. In the last two books are exact directions concerning all quantitative analytical determinations and the cautions to be observed in reading off the same.

It is clear, therefore, that quantities by weight, of other fluids, may be converted into equivalents of volume when we know their specific gravity. If, for example, one has 30 g. of glycerine to weigh out and knowing that the specific gravity of glycerine is 1.264, he needs to divide 1.264 by 30 to get the number of cc. of glycerine which will weigh 30 g. The division gives 23.7 cc. The following table will be useful in the reduction of weight measures to equivalents of volume, for some of the fluids used as microscopical reagents.

SPECIFIC GRAVITY OF FLUIDS AT 15° C.

Ethyl ether	0.736	Ammonia saturated	0.884
Alcohol absolute	0.794	Carbolic acid "	1.066
" 90 per cent	0.823	Chloroform	1.480
" 50 per cent	0.919	Acetic acid	1.055
" 40 per cent	0.940	Glycerine	1.264
Nitric acid	1.526	Hydrochloric acid	1.210
Carbon disulphide	1.271	Sulphuric acid	1.842
		Turpentine oil	0.870

Small variations of the temperature of the room may be entirely disregarded in the calculations.

III. APPLICATION OF THE VOLUMETRIC METHOD IN THE PREPARATION OF MICROSCOPICAL REAGENTS.

The volumetric[5] method may be successfully employed in the rapid preparation of certain reagents as Frey[6] has rightly asserted, and, on the other hand, it offers the possibility of determining with ease the per cent value of certain simple fluid reagents. We may here briefly set forth the few cases which concern the microscopist from the wide field of quantitative analysis. For the rest he may consult the work of Mohr already cited on page 277.

1. *Standard solution of oxalic acid.* Good commercial oxalic acid is pulverized and dissolved in a little warm water,

[5] Mohr, *l. c.*

[6] Frey, Mikroskop, p. 90.

so that still a considerable part of the acid remains behind on the bottom of the vessel. Then filter and crystallize by rapid cooling. The crystals should be drained off in a filter and dried at common temperature between blotting papers. To test their dryness press a piece of smooth paper upon them. If they are perfectly dry none of them will stick to the paper.[7]

Oxalic acid which has two molecules of water bound up with it has the chemical formula: $\begin{matrix} CO.OH \\ | \\ CO.OH \end{matrix} + 2\,H_2O$

The atomic weight is 126 (C = 12, O = 16, H = 1). Oxalic acid combines with two molecules of potassium hydroxide H K O to form potassium oxalate, $C_2O_4K_2 + H_2O$, a salt which contains one molecule of water, after this formula:

$$\left(\begin{matrix} CO.OH \\ | \\ CO.OH \end{matrix} + 2\,H_2O\right) + 2\,HKO = \left(\begin{matrix} CO.OK \\ | \\ CO.OK \end{matrix} + H_2O\right) + 3\,H_2O$$

Oxalic acid + 2 potassium hydroxide = potassium oxalate + 3 water. To form this salt the two molecules of potassium hydroxide, K_2O, contributes what corresponds to an equivalent of 94 (K = 39, O = 16). If now we reduce both numbers (126 and 94) to one atom of potassium by dividing by 2 we shall have the respective numbers 63 and 47.

We now weigh out 6.3 g. of pure oxalic acid on the chemical scales and dissolve it in exactly 100 cc. of distilled water. Each cubic centimeter of the solution will contain 0.063 g. of oxalic acid. With 0.047 g. of potassium added to each cubic centimeter of the oxalic solution we shall be able to transform it to potassium oxalate.

2. *Standard potassium solution.* It is necessary also to have a standard solution of potassium, that is, a solution of caustic potash in water, which contains in each cc. of the fluid 0.047 g. of the potassium dissolved. For this purpose prepare a pretty

[7] Fresenius, *l. c.*, p. 131, *f.*—Mohr, *l. c.*, p. 81, *f.*

concentrated solution of caustic potash in water, as it will be more particularly described under the title "potassium hydroxide." Transfer with the pipette any desired number of cubic centimeters of the fluid to a glass beaker and dilute with any desirable quantity of water. To this fluid add a few drops of litmus tincture till it appears evenly but distinctly blue.[8] Then let a standard solution of oxalic acid flow into it from a burette till the litmus tincture is uniformly changed to red. Now, suppose that in order to neutralize 5. cc. of our potash solution we should need to use 20.5 cc. of the oxalic acid solution. These 20.5 cc. contain 20.5×0.063 g. of oxalic acid which corresponds to 20.5×0.047 g. of potassium. This is contained in my 5. cc. of potassium solution. If I now dilute every 5. cc. of my solution of potash to 20.5 cc., every cc. of the solution will contain 0.047 g. of potassium and be a standard solution. I should add to each of my 5. cc. of potash solution 15.5 cc. of distilled water, or what is the same thing put into a 100 cc. flask 32.25 cc. of my potash solution, and fill up to 100 cc. with water. In regard to keeping solutions of potassium see below under "potassium hydroxide."

3. *Standard solution of sulphuric acid.* The potassium equivalent of 0.047 corresponds to a sulphuric acid equivalent of 0.040 for

$$\underset{\text{Sulphuric acid.}}{H_2SO_4} + \underset{\text{2 Pot. hydroxide.}}{2HKO} = \underset{\text{Potass. sulphate.}}{K_2SO_4} + \underset{\text{2 Water.}}{2H_2O}.$$

$SO_3 = 80$ ($S = 32$, $O = 16$) reduced to one atom of potassium equals 40. Let 5. cc. of pure English acid be put in a glass and diluted at will and colored with some drops of litmus tincture. Add now from the burette as much standard potash solution as will change the color to blue; read off, and dilute each cc. of the acid with as many cc. of distilled water as the number of cc. of potash solution used. One may employ the

[8] Digest for a long time in the water bath, 1 part commercial litmus with 6 parts water, filter, divide the blue fluid into 2 parts, neutralize the free alkali in the one-half, by repeatedly stirring it with a glass rod which has been dipped in very dilute nitric acid till the color appears red. Mix in then the other blue half and add one part strong alcohol and keep the tincture thus prepared in an open glass vessel, in some place free from dust. The tincture loses its color when kept in a closed vessel.

standard sulphuric acid solution in place of the oxalic acid the purifying of which consumes so much time.

It is necessary to know the equivalent number of the substances used in order to employ these standard solutions in preparing microscopical reagents.

EQUIVALENTS.

A. For Standard Solutions.

Sodium	0.03100	Barium chlorate (Anhydrate)	0.10405
Potassium	0.04711	Hydrochloric acid	0.03646
Ammonia	0.01700	Nitric acid	0.05400
Chromic acid (Anhydrate)	0.05024	Acetic acid	0.06000
Tartaric acid	0.07500	Oxalic acid	0.06300
Calcium chlorate	0.05546	Sulp. acid (Anhydrate)	0.04000

B. For $\frac{1}{10}$ Standard Solution.

Potassium iodide	0.016611	Potas. chloride	0.007457
Sodium chloride	0.005846	Silver	0.010797

The application of standard solutions to the preparation of microscopical reagents may be illustrated by some practical examples.

Examples for the application of the volumetric method in preparing microscopical reagents.

1. *To find the percentage of a given solution of potassium.* Transfer with the pipette 10 cc. of the solution to a glass cup, dilute with distilled water, add litmus tincture, and let standard sulphuric acid solution flow in till the color changes. It requires 75.5 g.

1 cc. acid = 0.047 g. alkali
75.5 cc. " = 3.5485 g. "

This 3.5485 g. of potassium is contained in 10 cc. of water. In 100 cc. (100 g.) there would be 35.485 g. The solution then contains 35.485 per cent of potash.

2. *To prepare a solution of a definite solution of potash, say 33.3 per cent.* Put 5. cc. of a partly concentrated solution of potassium in a glass, dilute with water, color with litmus, and add the necessary quantity of standard sulphuric acid solution = 58.4 cc.

1 cc. acid = 0.047 g. alkali
58.4 " = 2.7448 g. " in 5 cc. solution
Percentage = 2.7448 × 20 = 54.9
The proportion 33.3 : 100 : : 54.9 : x
$x = 164.86$

Therefore 100 cc. of our solution must be diluted to 164.86, or 10 cc. to 16.486 cc. to give us a 33.3 per cent solution.

3. *To prepare a 1 per cent solution of acetic acid.* Take 10 cc. of dilute acetic acid. Dilute with water and add litmus tincture. The necessary potassium solution for neutralization is 32. cc.

1 cc. potassium = 0.06 g. acetic acid
32 cc. " = 1.92 " "
1.92 × 10 = 19.2 = percentage
1 : 100 : : 19.2 : x $x = 1920$

To get 1 per cent solution, therefore, we must dilute 100 cc. of our tested solution to 1920 cc., or 1 cc. to 19.20 cc.

4. *English sulphuric acid of unknown strength to be diluted to a 20 per cent solution.* 1 cc. of acid requires 23.2 cc. of standard potassium solution for neutralization.

1 cc. potassium = 0.04 g. sulp. acid
23.2 " = 0.928 g.
0.928 × 100 = 92.8 = percentage
20 : 100 : : 92.8 : x, $x = 464$

So we must dilute 100 cc. of the acid to 464 cc., or 10 cc. to 46.4 cc. to get our 20 per cent solution.

5. *To test the correctness of a 5 per cent solution of chromic acid.* Chromic acid to be used as a microscopical reagent should have no foreign admixture. Most of all should it have no trace of sulphuric acid. Consequently its volumetric analysis should be accomplished by means of a barium chloride

solution. Make a standard solution of barium chloride ($Ba\,Cl_2 + 2\,H_2O$) by dissolving 12.2 g. of the crystals in 100 cc. of water, 1 cc. of this standard solution corresponds to 0.05024 g. of chromic acid. Put 25 cc. of the chromic acid solution to be tested in a glass, add a few drops of concentrated acetic acid and boil. Then add fluid ammonia which is free from carbonic acid till it feebly predominates and then heat. A Guy-Lussac bulb burette is filled with the standard solution of the barium chloride, and added drop by drop to the fluid which is being tested, that meanwhile being constantly shaken. There is produced a bright yellow precipitate, insoluble in hot water. The addition of the normal solution should cease when the yellow color of the fluid begins to grow indistinct. The reaction is ended at the moment when the yellow color disappears from the solution. It required 25.5 cc. of the barium chloride.

1 cc. of barium chloride = 0.05024 g. chromic acid
25.5 cc " " = 1.28 g. chromic acid
$25 : 1.28 :: 100 : x \quad x = 5.12 =$ per cent.

The solution is something more than 5 per cent. If an exactly 5 per cent solution is to be made it must be by the proportion

$$5 : 100 :: 5.12 : x$$
$$x = 102.4$$

So 100 cc. of the solution must be diluted to 102.4 cc.

IV. ENUMERATION AND PREPARATION OF MICROSCOPICAL REAGENTS.

A. INORGANIC COMBINATIONS.

1. Water H_2O.

It is always understood that the water used by the microscopist is distilled. It may be prepared in the well-known way or purchased of the druggist. In the latter case it should be carefully filtered before using. Potassium oxalate gives no trace

of a precipitate in pure distilled water even after long action, showing the absence of calcium salts. Should it be desired to use water free from carbonic acid, it should be boiled immediately before using.

2. Nitric Acid. HNO_3.

One should use the pure acid of the pharmacopœia which is perfectly colorless, has a stinging odor and develops a slight vapor in the air. It is used in a concentrated form, as well as diluted with water in various proportions as, for example, 50, 30, 10 per cent. The dilution may easily be made by the volumetric method. This, and the next following acids, should be kept in double-stoppered bottles (Fig. 126, p. 272), and on account of their development of fumes should be used under the microscope only with the largest cover-glasses.

Nitric acid is used as a medium for maceration with or without potassium chlorate (see p. 163), and with ammonia for reactions on nitrogenous and corky substances and middle lamella.

3. Sulphuric Acid. H_2SO_4.

Likewise only the pure, the so-called English acid, should be used either in a concentrated state, or in different dilutions with water, 1 volume of acid to 3 of water or 4 of water, specific gravity 1.20 or with greater quantities of water. It is used for dissolving cell membranes and cell contents; with iodine for testing cellulose, and with indol for membranes of wood cells.

4. Hydrochloric Acid. HCl.

(Muriatic Acid.)

As with the foregoing this acid should be employed only in the pure state, as it is used by the apothecary. It is perfectly colorless. The yellow tinted has been contaminated by combination with iron. It is used either concentrated, or in various dilutions. In the first case (cold saturated) it fumes in the air and like nitric acid should be used only with the largest

cover-glasses. By heating the concentrated acid to the boiling point (110°), a 20.2 per cent acid is produced which does not fume, and is recommended, for many microscopical purposes, as it has not been heretofore, to my knowledge. This acid does not affect the lenses in the least and should be used in all cases where the concentrated is not necessary.

Hydrochloric acid is a macerating and decalcifying medium (see p. 164), and serves as a test for proteids and calcium carbonate crystals and others.

5. Phosphoric Acid HPO_3.

(Metaphosphoric Acid.)

Forms an icy looking, glassy, solid substance (*Acidum phosphoricum glaciale*) which dissolves to a colorless fluid in water. As a microscopical reagent it is very little used.

6. Solutions of Iodine.

In botanic microscopical analysis, solutions of iodine are the reagents most frequently used. They are solutions of pure iodine[9] in different fluids, water, alcohol, glycerine, solutions of potassium iodide and of zinc chloride. They bear the names iodine water, iodine alcohol, iodine glycerine, potassium iodide of iodine, and chlor-iodide of zinc. The methods of preparing these various reagents are as follows.[10]

1. *Iodine water.* Iodine is soluble in water only in a very small quantity (1 : 0.00014) and forms a slightly brownish-yellow colored fluid. It is prepared by putting a small fragment in distilled water, the solution taking place in the course of a few days. A still better way is to prepare the solution immediately on the slide, by putting a small splinter of iodine in the

[9] For ordinary cases the crystalline commercial iodine will do. But if one prefers that which contains no trace of bromine he may prepare it by putting pulverized potassium iodide mixed with black oxide of magnesia in a small retort, and pour sulphuric acid over it. When the mixture is heated the iodine distils over and collects either in the neck of the retort or in a receiver.

[10] Harting, Das Mikroskop, p. 424, 475, *f.*, 499 — Harting, Entwicklungsgesch. des Pflanzenkeims, p. 35, 153, *f.* — Nägeli u. Schwendener, Das Mikroskop, p. 473, *f.* — Dippel, Das Mikroskop, Bd. I, p. 273 *ff.* — Frey, Das Mikroskop, p. 82, *f.* — Poulsen, Botanisk Mikrokemi, p. 1, *ff.*

water with the preparation under the cover-glass. The reagent works with more certainty when it is prepared immediately before using.

2. *Iodine alcohol.* Iodine dissolves readily in absolute alcohol and produces a deep brown fluid known under the name "tincture of iodine." For microscopical purposes we make the solution with a little excess of iodine, and afterwards dilute with absolute alcohol or distilled water. In the latter case some iodine will be separated. It is best to prepare it shortly before using.

3. *Glycerine iodine.* There are two modifications of this reagent in use.

(*a*) Pure concentrated glycerine to which is added a sufficient quantity of iodine, which dissolves slowly but abundantly and communicates a beautiful red brown color to the fluid. This may be used either in a concentrated state, or in different dilutions. The diluting medium may be either water or glycerine—the latter in case the object will bear the addition of no water.

(*b*) Dissolve, according to requirement, a greater or less quantity of potassium iodide in glycerine, and add to this solution, metallic iodine.

4. *Potassium iodide of iodine.* Three grammes of crystallized potassium iodide is dissolved in 60 cc. of distilled water and to this solution is added 1 gramme of metallic iodine. The resulting solution has a dark brown red color and can be diluted to suit by the addition of distilled water. For the investigation of certain objects, as lichen utricle,[11] the following composition is recommended. Iodine 0.06 g., potassium iodide 0.2 g., distilled water 16 g.

5. *Chlor-iodide of zinc.* Pure zinc in rods as it is used in the chemical laboratory is dissolved to saturation in muriatic acid. Still more zinc is added and the solution evaporated till it becomes a thick fluid. In this concentrated solution of zinc chloride dissolve to saturation potassium iodide, and then add a considerable quantity of metallic iodine which will slowly dissolve in the mixture (Schultz). Radlkofer[12] has given a more

[11] Poulsen, *l. c.*, p. 4. (German Trans. p. 6. English Trans. p. 6.)
[12] Dippel, *l. c.*, Bd. I, p. 274, *f.*

exact formula for the preparation of chlor-iodide of zinc solution. Evaporate a solution of zinc chloride prepared in the common temperature in a heat not exceeding that of boiling water, to a clear syrup of the specific weight of 2.0 and then dilute it with water to a specific weight of 1.8 which will require 12 parts water to 100 of the solution. In 100 parts of this fluid dissolve by gentle heat 6 parts of potassium iodide, and as much iodine as it will take up. The iodine solution of zinc chloride has now the consistency of concentrated sulphuric acid. It is perfectly clear and possesses a bright yellow-brown color.

Chlor-iodide of zinc solution — a very important reagent — may be suitably employed under the following modifications.[13]

(*a*) *A concentrated solution weak in iodine.* The zinc solution prepared according to Radlkofer's formula, concentrated and mixed with potassium iodide dissolves in the course of about 48 hours as much iodine as will give it a bright yellow-brown color. This modification can be used with those preparations which a stronger iodine mixture would color too intensely.

(*b*) *Concentrated solution strong in iodine.* This is produced by keeping the solution for several weeks in a dark place with an excess of metallic iodine. In the course of time it takes up so much iodine as to become of a dark red-brown color, holding a place somewhere about midway between glycerine iodine and potassium iodide of iodine.

(*c*) *Dilute solutions* may be obtained from *b* by the addition of various large quantities of potassium iodide which is dissolved in distilled water in the proportion of 1 : 20.

(*d*) For some purposes it is recommended to mix the iodine with the concentrated zinc chloride, under the cover-glass, with the preparation which is being examined. In that case the zinc solution should be used without the potassium iodide, and a little splinter of iodine added (Nägeli).

Hydriodic acid is formed in all iodine reagents by the influence of light. There are found traces of it, for instance, in iodine water after a few hours. It betrays its presence by an acid reaction.[14] Many reactions are interfered with by the presence

[13] Behrens in Flora, 1879, p. 239, in separate print, p. 58.

[14] Nägeli in Sitzungsber. d. Bayer, Acad., 1863, Bd. I, p. 484.

of hydriodic acid. In iodine water and iodine alcohol, which especially should be free from acid, its presence may be detected by litmus paper, or a less quantity by the following experiment. Placing a small quantity of starch on a slide, add some of the iodine to be tested and let it all dry up. Now if the solution contains no hydriodic acid the starch which was colored blue by the iodine will keep its color. If there is any hydriodic acid the starch will become yellow by drying. If we want to use perfectly pure solutions of iodine we must freshly prepare them each time. *All solutions of iodine are to be kept in the dark.*

Iodine reagents are useful in testing starch, cellulose and its modifications as well as the proteids.

7. Potassium Hydroxide K H O.
(Caustic Potash.)

It may be found in the market quite pure in snow-white sticks of a glassy crystalline fracture, dissolves in water with great eagerness and with the development of considerable heat. In a dry state the potassium acts quite indifferently towards the carbonic acid of the air, but in damp air it absorbs carbonic acid and water and is slowly transformed into potassium carbonate. The aqueous solution possesses the same quality in a high degree.

The presence of potassium carbonate in a potassium solution is easily detected by a sufficient addition of an acid, effervescence taking place in that case. In a potassium solution containing carbonic acid, baryta water produces a white precipitate, barium carbonate. As the presence of potassium carbonate works disastrously in many microscopical reagents the solution should be freshly prepared whenever it is to be used. For this purpose put the stick of potassium in a glass vessel, pour over distilled water and let it dissolve. As the potassium stick becomes clearer, that is as the layer of potassium carbonate is dissolved off, turn off the water and replace it with new. This second solution is the one to use. Keeping it but twenty-four hours it will absorb carbonic acid. Since the frequent

preparation of this solution is a waste of both time and money, I have constructed the following contrivance in which caustic potash solution may be kept for months or years without absorbing carbonic acid from the air.

A vessel containing the solution closed with a cork or glass-stopple will not accomplish this, since every change in the temperature will effect a change in the volume of air in the vessel by making it smaller or larger and so will cause a little of the outside air to find its way within. But if one allows the air free entrance and exit from the flask but removes the carbonic acid from it before it enters, the solution will always remain free from carbonic acid. The apparatus illustrated in Fig. 131 rests

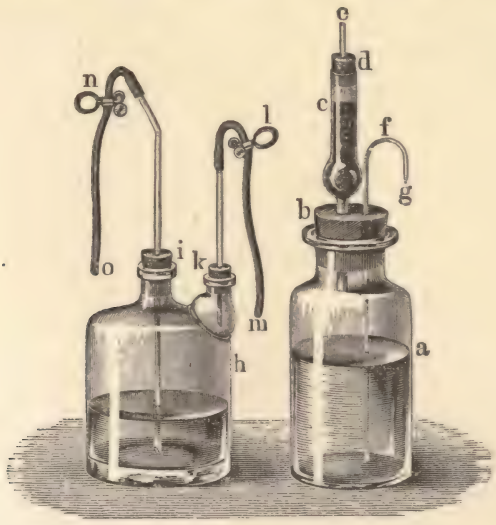


FIG. 131.

upon this principle. The wide-necked, clean and dry flask *a* is closed with a cork, *b*, perforated in two places, through which pass the tubes, *f* and *c*; *f* is a narrow glass tube which reaches almost to the bottom of *a*. At *f*, it is bent almost to a half circle and drawn out to a fine point at *g*; *c* is an ordinary straight calcium chloride tube closed at *d*, with a rubber stopper to exclude the dust, through which is thrust the small glass tube *e*, open at both ends. The calcium chloride tube must previously

be filled with a dry substance which will absorb carbonic acid. Graham has recommended for that purpose a mixture of Glauber's salts and quicklime. For the preparation of this, pound like parts of each in a porcelain mortar. Let the mixture effervesce fully and dry it in a tin dish over a free flame. Into the tube *c*, put a lock of glass wool to keep small pieces of the above described mixture from falling through. Then fill the tube with the absorbing mixture which should not be a powder but small pieces. Then put a thin layer of wax over the cork stopple and over this paint a coat of asphalt varnish. After the varnish is dry the bottle is to be filled with the solution of caustic potash freshly prepared by the above described process. Before the filling takes place blow in at *e* till the air having carbonic acid in it is all driven out. Then dip the end of the tube *g* in the solution, and suck at *e* till the bottle is filled. Then put over the end of *g* a little piece of thick walled rubber tube with a piece of glass rod filling and closing the end of it. So arranged, the apparatus can stand for months long and not absorb the least trace of carbonic acid. If I want a drop of the solution on the slide, I remove the cap from *g* and put it over the tube *e*, and then placing my hand on the part of the flask *a*, which contains the air, the air will be warmed and expanded, and consequently will push out through the narrow tube *g*, the required quantity of the alkali. Should it be desired to transfer larger quantities of the potassium from *a*, into another vessel, we may connect it with a common wash bottle which is half full of the ordinary solution of potash. Then remove the spring clips, *l* and *n*, shove *m* down over *e* and blow in *o*, till the desired quantity of the alkali has been driven out through *g*.

Potassium hydroxide is used in alcoholic as well as in aqueous solution. The preparation of the former (Russow's potassium alcohol) has already been described on p. 200. The aqueous solution is used both in its concentrated and dilute forms, the alkaline element being gauged to meet the resistance of the particular object tested. The quantity of potassium in the solutions can easily be determined by the volumetric method.

There are manifold uses for potash solutions in microscopy. Besides its use for bleaching (pp. 199, 200), it has a reaction for

protoplasm, cork substance, tannic acid, crysophanic acid, sugar, etc., etc.

8. Potassium Chlorate $KClO_3$.

This is a small, whitish, greasy-looking crystal found sufficiently pure in the market and used chiefly in the preparation of maceration mixtures (see p. 163).

9. Potassium Nitrate KNO_3.

Its use in botanical microscopy is extremely limited. It is procured pure most easily by neutralizing a solution of potassium with nitric acid and crystallizing the resulting salt.

10. Potassium Bichromate $K_2Cr_2O_7$
(Potassium pyrochromate.)

It can be got sufficiently pure by taking the larger crystals of the common salt, washing out, and recrystallizing three or four times. Dissolved in water it is used for hardening tissue (see p. 178), and as a test for gum and tannic acid.

11. Sodium Chloride NaCl.
(Common Salt.)

Common cooking salt contains numerous foreign substances. Its preparation for our uses is best done from pure potassium carbonate (or bicarbonate) by mixing with hydrochloric acid and crystallizing. It is used but rarely and in very weak solutions.

12. Ammonia $(NH)_4HO$.
(Ammonia hydroxide.)

This is frequently used in saturated aqueous or dilute solutions. It is sold in the drug shops under the name of Liquor ammonia caustica. If one wishes to prepare it for himself he may do it with sal ammoniac and quicklime, washing the developed ammonia gas in a small water flask in strong potash or soda lye,

and leading thence in a double bent tube to the absorption flask filled with cold distilled water, carrying the end of the tube down very near to the bottom of the flask. The absorption flask should be set in cold water. The fluid is saturated with the ammonia when bubbles of the gas rise through it.

Ammonia may many times be used in microscopy in place of caustic potash. It comes into use also in Hanstein's method of bleaching (see p. 199). With nitric acid it is a test for proteid combinations.

13. Ferric Chloride $Fe Cl_6$

For sale by the apothecary under the name of Liquor ferri sesquichlorate. The aqueous solution is employed in microscopical analysis in not too dilute a state for a test of tannic acid. The combination can also be obtained by dissolving iron in nitro-muriatic acid (Aqua regia = I Vol., $H N O_3$ + IV Vol., H Cl). After evaporation the ferric chlorate remains as a greenish mass sometimes also as crystals.

14. Chromic Acid $Cr O_3$

(Anhydrated chromic acid.)

It can usually be had sufficiently pure, as beautiful red precipitate crystals, in the market. It easily dissolves in water with brown, and in dilute state, yellow color. For microscopical purposes chromic acid should contain no sulphuric acid (see p. 282). It is tested by the so-called Hepar-test.[15] Chromic acid is used in solutions of various strength, 1 part acid and 6 parts water for studying the layers of starch grains (Dippel), also a one per cent solution as a hardening medium. The degree of concentration of the chromic acid solution may be determined by the volumetric method. The solid acid should be kept dry in a well closed glass vessel.

[15] Of the chromic acid which is supposed to contain sulphuric acid, make a pretty strong solution and add to it baryta water, barium chromate, and if there be sulphuric acid present barium sulphate will be precipitated. Filter and wash the precipitate and put some of it on charcoal and melt it down with soda.

Put the calcined mass when cold on a silver piece and moisten it. If sulphuric acid be present, the silver surface will be blackened or turned a dark yellow by the formation of silver sulphates at the point where the mass rests.

15. Copper Sulphate $Cu\,S\,O_4 + 5\,H_2O$
(Blue vitriol.)

A sufficiently pure preparation for most purposes may be made by crystallizing the commercial blue vitriol three or four times. A purer article will be obtained if we add ammonia fluid to a concentrated aqueous solution of this salt, from which cupric hydroxide will be precipitated ($Cu\,O,\,H_2O$). Then wash till the wash water shows no trace of cloudiness when baryta water is added. Then dissolve the precipitate in dilute sulphuric acid and evaporate to crystallization and we have the pure sulphate. It is used mostly in a concentrated, rarely in a dilute, aqueous solution.

Copper sulphate is used in connection with potassium hydroxide as a test for cane and grape sugar, dextrine, proteid substances, etc.

16. Cuprammonia. $Cu\,2\,(N\,H_4)\,O_2$
(Copper oxide ammonia. Schweitzer's Reagent.)

This reagent was discovered in 1857 by Schweitzer. It forms a beautiful blue fluid which easily decomposes, especially in the light, and is produced by a combination of ammonia oxide and copper oxide. The reagent has been prepared by different methods, the more important being the following.

Schweitzer[16] prepared the solution used by him in the following way. "I prepare the basic-hyposulphate of copper oxide described by Heeren by a careful precipitation of a solution of hyposulphate of copper oxide by means of a dilute ammonia fluid, filtering and washing the bright green precipitate. Then I put this combination still damp into concentrated liquid ammonia. It easily dissolves with the development of heat, but on cooling crystals of hyposulphurous-copper oxide ammonia are formed from the solution. Together with this cuprammonia must have been formed by the dissolving of the basic salt in ammonia, and this must be contained freely dissolved

[16] Journal für prakt. Chemie, Bd. LXXII (Leipzig, 1857), p. 109, *ff.*

in the dark blue fluid from which the crystals have been separated."

Böttcher[17] used a glass tube about two feet long and one to two inches wide, open at the top and narrowed at the bottom, terminating in a rubber tube provided with a spring clip. This tube was loosely filled with strips of copper rolled thin and then placed upright in a holder and filled with strong ammonia fluid. After a few minutes this was permitted to run off into a vessel set to catch it, and again turned back over the copper and so on for some hours. Thus in a relatively short time one may get a deep dark blue fluid perfectly saturated with copper oxide.

Neubauer[18] used for preparation a copper oxide which was precipitated by the presence of sal-ammoniac from copper vitriol solution with soda lye. The precipitate should be thoroughly washed first by decantation and at last by filtering and then kept damp under water. For the preparation of the reagent it is shaken up in an excess of ammonia as long as any will dissolve in it. The result is a deep blue solution.

According to Wiesner[19] the cuprammonia solution is prepared by turning 13–16 per cent ammonia water over a quantity of copper chips in an open flask and letting it stand.

I have employed another method similar to that of Neubauer, which gives a very efficient and pure preparation. If we precipitate cupric sulphate with caustic soda or potash, there will commonly form, corresponding to the carbonic acid contained in the alkali, traces of basic cupric carbonate ($Cu_2 C O_5 H_2$), in the form of a blue green precipitate which is soluble in ammonia. On the other hand a cuprammonia is more effective, for which the cupric hydroxide has been precipitated with an ammoniacal salt mixed with a solution of cupric sulphate. The next following method of preparation prevents the first unfavorable result just now named, and furnishes an intensely efficient reagent. Dissolve 2 g. of quite pure crystallized cupric sulphate in 100 cc. of distilled water and to the solution add a few

[17] Böttcher in Neues Repert. für Pharmacie, Bd. XXIII, p. 732.

[18] C. Neubauer in Zeitschr. für Analytic. Chemie von Fresenius Jahrg. XIV (Weisbaden, 1875), p. 196.

[19] J. Wiesner, Concerning the influence of cuprammonia on animal tissue and tissue elements (Sitzungsber. d. math.-naturw. Cl. d. K. Acad. d. Wiss., Wien, Bd. XLVIII. Abth. II, 1863, p. 199, *ff*).

drops of concentrated chlorammonia solution. Then prepare a weak solution of potash with 1 g. of caustic potash to 100 cc. of distilled water to which add a little baryta water by which any potassium carbonate which is present may be transformed to barium carbonate and thrown down as a white precipitate. The two solutions are then to be poured together, and the cupric hydroxide will precipitate, from which, when it has settled, the water is to be turned off and distilled water substituted and this again repeated several times, and finally filtered. The washing of the precipitate must necessarily go on till the wash water shows no white precipitate when baryta water is added. The still damp cupric hydroxide is put in a suitable vessel and a little concentrated fluid ammonia poured over it till all of the hydrate is dissolved; then, finally, filter through glass wool. By this means the barium salts insoluble in ammonia (barium sulphate and carbonate) are separated out and are left behind, also commonly a small quantity of the cupric oxide (Cu O).

It is better to use the reagent when freshly prepared. At all events it must be kept in the dark since, as remarked, it quickly decomposes in the light. It serves as a test of cellulose.[20]

17. Mercuric Chloride. $HgCl_2$.
(Corrosive sublimate.)

It may be had in the market sufficiently pure in little white sparkling crystals and should be used in very dilute aqueous or alcoholic solutions, for example, 1 part mercuric chloride and 100 parts water, or 2 parts of the sublimate to 100 parts of alcohol (Pfeffer). Dippel[21] used a still more dilute solution of the sublimate in the study of protoplasm, 1 part sublimate to 500 parts of water.

[20] For the same purpose one may use, even if less advantageous, a solution of basic cupric sulphate in ammonia, also of basic cupric carbonate in ammonia, finally, also, nickel oxide ammonia. (Schacht).

[21] Dippel, Mikroskop, Bd. I. p. 282.

18. Millon's Reagent (Liqueur nitromercurique.)

$$Hg_2\,2\,N\,O_3 + Hg\,2\,N\,O_3 + H\,N\,O_3.$$

The preparation of this reagent (quicksilver and quicksilver nitrate) in an acid solution is accomplished, according to its discoverer E. Millon, in the following manner.[22] Pour upon the pure metal an equal weight of nitric acid which contains $4\frac{1}{2}$ equivalents of water. The reaction immediately begins in a lively way in the cold. When it begins to slacken warm gently till the metal is fully dissolved. But directly it is finished add two volumes of distilled water to one of the quicksilver solution. After some hours decant the fluid part which stands over the crystalline mixture of mercuric and mercurous nitrate. This fluid reacts cold upon albuminous substances — its reaction is complete, however, only at 60° to 70°. It is well to boil the mixture itself. A continual contact with the reagent does not change the red matter. It should be remarked that neither in the mercuric or mercurous nitrate, nor in their mixture is to be sought the reagent alone. It is necessary that, to a solution containing these two salts, nitric acid should be added before coloring will be produced. The pure mercuric nitrate which is saturated with nitric acid reacts similar to, but not so well as, a saturated nitric acid mixture of the two salts.

According to Hartig[23] the mercuric and mercurous nitrate can be prepared by dissolving mercury in a like weight of foaming nitric acid, the solution afterwards to be mixed with equal parts, by volume, of water. The reagent should be used only with the largest cover-glasses since it throws off acid fumes. It is principally a test for albuminous substances.

19. Osmic Acid $Os\,O_4$.

(Perosmic acid.)

Osmic acid, or perosmic acid as it is commonly named in microscopy, forms colorless, sparkling, needle-like crystals, and

[22] E. Millon, Sur unréatif propre aux composés pratiques (Annals de Chemie et de Physique, IIIe Sér., tome XXIX, 1850, p. 507, *ff.*

[23] Hartig, *l. c.*, p. 154.—Cfr. also Dippel, *l. c.*, Bd. I, p. 281, Poulsen, *l. c.*, p. 29, *f.* (trans. p. 38). Nägeli and Schwendener, *l. c.*, p. 475.

should be used in a very weak aqueous solution. The solution of this expensive reagent (1 g. costing 8 to 10 M.) has a very disagreeable smell, like chlorine, and the vapor from it attacks the eyes and the mucous membrane of the nose in a very unpleasant way. The strength of the solution varies between 1, and 0.1 per cent. Poulsen[24] recommends a mixture of 9 parts 0.25 per cent chromic acid with 1 part 1 per cent osmic acid for the preparation of young meristem tissue.

Osmic acid has a manifold application as a microscopic reagent for testing fats and oils and for the study of protoplasm and nuclei (Strasburger), and for hardening cell contents.

B. ORGANIC COMBINATIONS.

20. Alcohol (Ethyl Alcohol) $C_2H_5.HO$.

It is used in the anhydrous state (absolute alcohol) as well as diluted in various degrees with water and with glycerine (p. 178) and with potassium (p. 200). It can be purchased sufficiently pure. It has an extended usefulness for hardening cell walls and cell contents (p. 178) and as a test of asparagin, inulin, etc.

21. Ether (Ethyl Ether) $(C_2H_5)_2O$.

Commercial ether (sulphuric ether) has, as such, an abundant application, for drying preparations which are to be mounted in Canada balsam (p. 222) and for dissolving resins, fats, and essential oils in plant cells.

22. Acetic Acid $C_2H_4O_2$.

(Glacial acetic acid.)

The concentrated, or the so-called "glacial" acetic acid must be considerably reduced before it can be successfully used, 1 vol. acid to 2, 3, or 4 volumes of water. Very weak (1 per cent) aqueous solutions are useful in the study of nuclei (Stras-

[24] Poulsen, *l. c.*, translation p. 20.

burger). Acetic acid was also applied by Hanstein for bleaching (see p. 199), for tests of oxalic and carbonic acid salts and for making nuclei visible.

23. Cupric Acetate $Cu(C_2^{\cdot}H_3O_2^{\cdot})_2$.

Quite pure acetate of copper can be found in the market. It may be purified if necessary by repeatedly recrystallizing. The saturated aqueous[25] solution is employed in microscopy and is used as a test of turpentine resin.

24. Sodium Nitro-Prussiate.

$Na_2 Fe C_5 N_5 (N O) + 2 H_2 O$.

We use the commercial crystallized salt which must be kept in an air-tight bottle. It should be prepared fresh on the rare occasions when we shall want to use it as a test for free sulphur in bacteria, for example.

25. Potassium Ferrocyanide $K_4 Fe (C N)_6$.

(Yellow prussiate of potash.)

Will be used in rare cases in an aqueous solution as a test for iron. It may be replaced also by an alcoholic solution of potassium sulphocyanide (CN. S K), (Poulsen.)

26. Oxalic Acid $C_2 H_2 O_4$.

The commercial crystallized acid may be suitably purified by recrystallization. In its aqueous and alcoholic solutions it comes into frequent use as a stain for sections as will be shown later.

27. Asparagin $C_2 H_3 (N H_2) < \begin{matrix} COOH \\ CO.NH_2 \end{matrix}$

Borodin[26] used a concentrated aqueous solution of asparagin

[25] Franchimont in Archives Neerlandaises, t. VI, 1871, p. 427.
[26] Borodin in Botan. Zeitung, 1878, p. 804.

as a test of asparagin crystals in etiolated vegetable tissue. According to Poulsen,[27] asparagin is best prepared by the evaporation of the boiled and filtered sap of young etiolated leguminous sprouts (*Lupinus*), or by evaporating the dialyzed aqueous extract of altheæ root. The asparagin then crystallizes out.

28. Cane Sugar $C_{12}H_{22}O_{11}$.

This substance may be properly used in microscopical analysis in the form in which it appears in the pharmacopœia under the name of *Syrupus simplex*. It serves, as Raspail first found, as a test with sulphuric acid for proteids.

29. Aniline Coloring Matter.

Recently numerous aniline colors have been recommended for staining sections of plant tissue, after being first introduced by Hanstein as histological reagents in microscopical botany. Now a large number of aniline colors is prepared as staining media. For example, aniline green (methyl green), methyl violet, fuchsin, iodine green, aniline brown, bismark brown, saffranin, magdala, gentian violet and others. We shall not, however, give all the proposed methods of staining. Many of them, especially such as have been discovered in England and America, are not suitable for a scientific work but offer the means only of a very pretty pastime, since they do indeed give to a section under examination a very beautiful color, but do not differentiate any histological relations. Whoever desires recipes for these beautiful colors may look for them in microscopical journals written in English, which commonly swarm with them. We mention here only:

1. *Hanstein's aniline mixture*.[28] Hanstein's method of aniline staining is to this day still the most useful. His aniline mixture is prepared in the following manner. Take like parts

[27] Poulsen, *l. c.*, p. 34, Trans., p. 45.

[28] Hanstein in Sitzungsber. d. natur. Ver. der pr. Rheinland u. Westfalens, 1868. — Bot. Zeitung, 1868, p. 708, *ff.*

of aniline fuchsin and methyl violet,[29] pulverize and intimately mix, till they form a cloudy violet powder. Dissolve as much of this mixture in absolute alcohol as will produce a concentrated solution, which appears in the glass flask where it is kept perfectly black with a purple metallic luster. For most purposes this concentrated solution is used direct. For many highly colored tissues instead of this a solution diluted with alcohol. The degree of dilution may be chosen according to the nature of the object stained. The dilute solution should contain as much aniline as one can see through without difficulty when put in a test tube of average caliber.

The methods of staining in all aniline mixtures are essentially the same. They will now be briefly pointed out.

(*a*) Pour a few drops of the aniline solution into a watch-glass and transfer the section from absolute alcohol to it for a few moments, as many as necessary to produce the best effect, holding it fast with the forceps. Then dip it repeatedly in absolute alcohol till all adhering aniline disappears and transfer it to the slide into water or glycerine.

(*b*) Transfer the section from the alcohol to the slide and with blotting paper or the like, remove all superfluous alcohol, not so, however, that the section becomes dry. Now give it a drop of the aniline solution (nearly or quite concentrated), and let the section so far dry, that it is only just damp. Then flow the section with alcohol till all adhering aniline is dissolved and washed away, letting the flowing alcohol run off the obliquely held slide into a vessel prepared for it. After washing add a drop of water or glycerine and put on the cover-glass (orally communicated to me by Hanstein).

2. *Aniline fuchsin solution.* It is prepared by dissolving aniline fuchsin in absolute alcohol,[30] or in like parts of water and alcohol.[31] The latter should be used to test corky membranes.

3. *Frey's fuchsin solution.*[32] It consists of 1 g. crystallized fuchsin, 20 to 25 drops absolute alcohol and 15 cc. of water.

[29] Or two parts aniline fuchsin and one part methyl violet.
[30] Poulsen, *l. c.*, p. 40, trans., p. 59.
[31] Oliver in Bull. de la Soc. bot. de France, t. XXVII, 1880, p. 234, *f.*
[32] Frey, *l. c.*, p. 97.

It may be applied to plant tissue for comparison with the effect produced by Hanstein's aniline. The corresponding blue fluid is prepared by dissolving 0.02 g. aniline blue in 20 to 25 drops absolute alcohol and 25 cc. of water.[33]

4. *Methyl violet of Koch.*[34] A few drops of a concentrated solution of methyl violet in absolute alcohol added to 20 cc. of distilled water, thereby producing an intensely yellow colored fluid. This fluid is used for coloring bacteria (see below) and after the staining is ended the preparation should be washed with water or a dilute solution of potassium acetate. For the same purpose an aniline brown is recommended.

5. *Methyl green*, in a not too concentrated solution with (Strasburger), or without (Treub) 1 per cent acetic acid colors nuclei and chlorophyll grains (Hanstein), green.

6. *Picro-aniline.*[35] Two to three cc. of a saturated solution of aniline blue (bleu de nuit) are mixed with 50 cc. of a saturated solution of picric acid (especially good for animal tissues not tested by me).[36]

30. Aniline Sulphate $2\,C_6H_7N.\,SO_4H_2$.

This substance which can be had in the market is a violet brown powder. It is used in a concentrated solution.[37] It is advantageous to acidulate this solution somewhat with sulphuric acid. It may be kept for years without losing its virtue. It colors cell walls containing liguin gold-yellow.

According to Wiesner[38] the aniline sulphate of the market is pure enough, while von Höhnel[39] contradicts this and uses instead muriatic aniline. This he dissolves in water and strongly

[33] Frey, *l. c.*, p. 98.

[34] Koch in Cohn's Beitr. z. Biologia d. Pfl. Bd. II, p. 406.

[35] Bachmann, Leitf. z. Anfert mikr., Danerpräparate, München, 1879, p. 28.

[36] Cf., also W. Sterling in Journ. of Anat. and Physiol., Vol. XV, 1881, pp. 349-354, which proposes numerous complicated staining media, viz., picro-carmine and aniline, picro-carmine and iodine green, eosin and iodine green, gold chloride and aniline (not all tested). Cf. further, Journal of the Royal Microscopical Soc., Ser. II, Vol. I, p. 527, *ff.*, finally, *l. c.*, p. 868, where Richardson proposes to color vegetable tissue with Atlas-scharlach, solution of aniline blue, iodine green, and malachite green.

[37] Burgerstein in Sitzungsber. d. K. K. Akad. d. Wiss. Wien, Bd. LXX, 1 Abth., 1874, p. 341.

[38] Wiesner, The same publication, Bd. LXXVII, 1 Abth., 1878, p. 60.

[39] v. Höhnel, The same publication, Bd. LXXVI, 1 Abth., 1877, p. 527.

acidulates with hydrochloric acid. The alcoholic muriatic aniline solution with the subsequent addition of water is also recommended.

31. Phenol (Carbolic acid) $C_6H_5.OH$.

This acid alone is not often used, but in connection with hydrochloric acid (phenolhydrochloric acid), it has been recently proposed by v. Höhnel[40] as a test for wood substances.

The phenolhydrochloric acid of v. Hohnel is prepared by making a concentrated solution of crystallized carbolic acid of the utmost possible purity in concentrated hydrochloric acid. The carbolic acid is dissolved in the least possible amount of muriatic acid by the application of heat, and while it is cooling as much more muriatic acid is slowly added as will clear up the existing cloudiness.

32. Phloroglucin (Trioxhydrobenzol) $C_6H_3.(OH)_3$.

This substance widely distributed[41] in the vegetable kingdom is in a pure state a small, bright yellow, transparent crystal soluble in both water and alcohol. Phloroglucin forms at the present time one of the best reagents we have for testing lignified cell walls. The following sketch of the discovery of the reagent will not be uninteresting.

V. Höhnel[42] having put a section of a twig of *Salix purpurea* in hydrochloric acid observed that the woody membranes of the section was colored an intense violet. He ascribed the coloring to a substance frequently occurring in the vegetable kingdom and which he called xilophilin and whose presence he detected in 143 species of plants. Wiesner[43] afterwards more exactly investigated the xilophilin of Höhnel and found it to be a mixture of phloroglucin and pyrochatechin. It further ap-

[40] v. Höhnel, *l. c.*, p. 528, p. 700, *ff.*

[41] Th. v. Weinzierl. Concerning the distribution of phloroglucin in the vegetable kingdom (Oesterr. Bot. Zeitschr., 1876, p. 285–304).

[42] F. v. Höhnel, Histo-Chemical investigations of xylophilin and coniferin. Sitzungsber. K. K. Akad. Wein, Bd. LXXVI, 1 Abth., 1877, pp. 663–716.

[43] J. Wiesner, Note concerning the action of phloroglucin and some related bodies upon lignified cell membrane (last cited publication, Vol. LXXVII, 1 Abth., 1878, pp. 60–66.

peared that phloroglucin was a most sensitive reagent on wood substances, and that commonly lignified tissue treated to hydrochloric acid served as a most sensitive reagent for phloroglucin.

Phloroglucin is used in an aqueous or alcoholic solution. Wiesner recommended a 9 per cent, but still gave also a 0.01, 0.005, even 0.001 per cent solution as capable of making a reaction.

Since phloroglucin is a very expensive article at this time, and not to be had by every one,[44] a cherry-wood extract containing phloroglucin may be used, which is prepared in the following manner.[45]

Not too small branches of cherry tree are washed and fastened into a bundle and then by means of a plane cut into fine chips or shavings. Pour alcohol over these letting it stand twenty-four hours to extract the chlorophyll, which much disturbs the action of the reagent, and for this reason not too young twigs should be used on account of their having so much chlorophyll. Turn off the first and replace with a fresh quantity of alcohol, and let it stand for several days, frequently stirring. Then filter the extract and evaporate the fluid almost entirely away, till a piece of blotting paper which has considerable wood fibre in it, dipped first in hydrochloric acid and then into the solution turns rapidly and intensely violet. In this way one gets a brown liquid which smells like camphor.

33. Indol $N C_8 H_7$.

Niggl[46] has recently recommended this very unpleasant smelling stuff[47] as a reagent for lignified cell membranes. It cannot be used in an alcoholic solution since it spoils in a few days. It is but little soluble in water and one can work for months with a few little crystal plates for a solution. The best way of making the solution is by warming the water. It should be em-

[44] To be had of Dr. Theodor Schuchardt, Görlitz, Schlesien Germany. Also of J. T. Brown, cor. Washington and Bedford Sts., Boston, Mass. Mr. Brown also will keep on hand all the micro-chemical reagents.

[45] v. Höhnel, *l. c.*, p. 685.

[46] M. Niggl. Indol a reagent on lignified cell membranes. Microchemical investigations (Flora, 1881, pp. 545–559, pp. 561–566).

[47] Beyer in Ann. Chem. Pharm., Bd., CXL, p. 1, *ff*, p. 295, *f*.

ployed with sulphuric acid of the specific gravity of 1.2, which consists of 1 vol. English sulphuric acid diluted with 4 vol. water (*cfr.* p. 236).

34. Eosin $C_{20} H_8 B_4 O_5$.

In a weak aqueous solution this reagent, which may be had free from arsenic in the market, is used according to Poulsen[48], to color bacteria, also according to this author it colors dead protoplasm a rose-red excellently well. It has recently been recommended for double staining of the tissue of the higher plants, the methods of which require further testing.[49]

35. Hæmatoxylin $C_{16} H_{14} O_6 + 3 H_2 O$.
(Extract of Logwood.)

This reagent may be had perfectly pure in the market. Bohmer introduced it into histology. Frey[50] gives the following formula for the preparation of hæmatoxylin in solution. Dissolve 1 g. of the coloring matter in absolute alcohol. Then prepare an alum solution of 0.5 to 1 g. in 30 cc. distilled water. Into this drop the alcoholic solution till it has attained a deep violet color. The fluid should now be allowed to stand some days in the air and then filtered; also, afterwards it must be filtered from time to time. Duration of staining process from 5 to 30 minutes. Wash with distilled water. Over colored preparations may be bleached by putting them in a solution of alum.

According to Poulsen[51] 0.35 g. hæmatoxylin should be dissolved in 10 g. water and to this should be added a few drops of an alum solution which consists of 3 g. alum and 30 g. of water.

It may be added that hæmatoxylin colors the more intensely the more alum it contains, but at the same time the section is made more brittle.

[48] Poulsen, Om nogle mikroskopiske Plantorganismer; Nat. Foren. vidensk, Medd· Köbenhavn, 1876-80, p. 235 (separatabz, p. 7). Botanisk Mikrokemi, p. 89 (Trans. p. 57).

[49] Amer. Monthly Microscop. Journal 1880, p. 81, *ff.*

[50] Frey, *l. c.*, p. 99.

[51] Poulsen, *l. c.*, p. 98, translation, p. 56.

Poole[52] makes a double staining of vegetable tissue with hæmatoxylin and a dilute aniline solution.

For animal tissue Frey,[53] after Stralzoff, recommends a double staining with hæmatoxylin and an ammoniacal carmine solution (see p. 306), which method may be applied, probably unchanged, to vegetable tissue. The section should be stained with hæmatoxylin washed with distilled water, and then laid in the carmine fluid; after that it should be again washed, and finally subjected to the influence of a weak solution of alum. It does not keep well in glycerine. According to Schmitz,[54] preparations hardened in picric acid are especially adapted to staining with hæmatoxylin. Hæmatoxylin colors nuclei a deep blue,[55] and may be applied to the staining of bacteria.[56]

36. Cochineal Extract.

An aqueous extract of the pulverized insect prepared with heat contains carmine acid ($C_{17}H_{18}O_{10}$), and is useful in staining vegetable tissue. It is very liable to mould and so must be protected by a few drops of carbolic acid. Before using, a few drops of acetic acid or solution of alum should be added.

Czokor[57] has recently recommended a cochineal carmine solution which is notable for being capable of preservation unchanged for a long time. Triturate 1 g. cochineal with 1 g. burnt alum to a fine powder. Then add 100 cc. of distilled water and boil till it is but 60 cc., cool, add a few drops of carbolic acid and filter several times. The resulting solution is a beautiful carmine color and may be kept without change for six months. Then add again a little carbolic acid and filter.

The cochineal colors bast elements, also many wood cells, proteid bodies and cell nuclei.

[52] Poole. in Quart. Jour. of Micros. Science, New Series, Vol. XV, 1875. p. 375, *ff.*
[53] Frey, *l. c.*, p. 101.—*Cf.* further Brandt in Biolog. Centralbl., 1881, p. 202. *ff.*
[54] Schmitz in Sitsungsber. der niederrh. Gesellsch., Bonn, 1880, Jahrg. XXXVII, p. 160.
[55] Johow, The cell nuclei of the higher monocotyledons, Bonn, 1880, p. 9.
[56] Koch in Cohn's Beitr. z. Biol. d. Pfl., Bd. II, p. 421.—Poulsen, *l. c.*
[57] Joh. Czokor in Archiv für mikrosk., Anatomie, Bd. XVIII, 1880, p. 412, *ff.*

37. Carmine Solutions (Carmine red $C_{11} H_{12} O_7$).

Commercial carmine is the coloring matter with which it was first attempted (by Th. Hartig) to stain anatomical preparations. Hartig is also the inventor of the method of staining as mentioned on p. 267. Since the introduction of this staining medium, many naturalists have employed various mixtures of which the most important are the following.

1. *Hartig's ammoniacal carmine.*[58] Commercial carmine is mixed with water and ammonia fluid is added in drops till a perfect solution results. The solution should then be filtered, and by very gentle heat evaporated to dryness. This carmine ammonia thus prepared may be dissolved in water and can be kept for years in good condition as an aqueous solution.

2. *Gerlach's ammonium-carminate.*[59] According to Frey,[60] this is prepared best in the following way. Take 0.2 to 0.4 g. of carmine, mix with 30 cc. of water and add a few drops of ammonia. Thus a part of the carmine will be dissolved and the fluid should be filtered. The rest which remains behind may be kept over for future use. If the filtrate smells at all strongly of ammonia it should be permitted to evaporate for half or a whole day under a glass bell. If after the lapse of time grains of carmine begin to be deposited a drop of ammonia will restore the solution. In order to get any desired color from this mass it should be transferred to water, drop by drop, the color growing of course from a light to a darker and more intense red.

3. *Frey's glycerine carmine.*[61] Dissolve 0.2 to 0.4 g. of carmine in the required amount of ammonia and add 30 cc. of distilled water. To the filtered fluid add 30 g. of good glycerine and 8 to 11 g. of strong alcohol. The tincture should be used unmixed, or with a further addition of glycerine.

4. *Thiersch's oxalic acid carmine.*[62] One g. carmine is dis-

[58] M. Hartig, Entwick'ungsgesch. d. Pflanzenk. p. 154. — Dippel, *l. c.*, Bd. I, p. 284.— Poulsen, *l. c.*, p. 36, trans. p. 49.

[59] Mikrosk. Studien aus d. Gebiete der menschl. Morphologie, Erlangen, 1858.

[60] Frey, *l. c.*, p. 93.

[61] Frey, *l. c.*, p. 94.

[62] Frey, *l. c.*, p. 94.—Dippel, *l. c.*, Bd. I, p. 285.—Poulsen, *l. c.*, p. 36, *f.*, trans. p. 50. Bachmann, *l. c.*, p. 62.

solved in 1 cc. of ammonia, and mixed with 3 cc. of distilled water. Also dissolve 8 g. crystallized oxalic acid in 175 cc. of distilled water. Then mix the solutions, add 16 cc. of absolute alcohol and filter. If the solution has an orange color which comes from a predominance of the oxalic acid, it can be corrected by carefully adding drops of ammonia. If there are in addition deposits of crystals of ammonia oxalate in the filtrate, which may happen from the addition of the ammonia or the alcohol, the fluid must be filtered the second time; subsequent occasional filtering is also beneficial. The tincture stains very quickly. The coloring matter which adheres to the object should be washed off with 80 per cent alcohol. If the color becomes too dark or diffuse, soak the preparation out in an alcoholic solution of oxalic acid.

5. *Thiersch's borax carmine.*[63] Dissolve 2 g. borax in 28 cc. distilled water and add 0.5 g. pulverized carmine. To the red solution thus produced add 60 cc. of absolute alcohol and filter. (If on the filter paper there remains a mixture of undissolved carmine and borax it may be dissolved in distilled water and kept over for future use.) For soaking out use an alcoholic solution of oxalic acid, or boracic acid. The mixture colors somewhat slowly but very beautifully. According to Frey (*l.c.*) one gets the most beautiful coloring when one lays the preparation for a moment in the solution after it has been previously impregnated with boracic acid. Studies of vegetable nuclei are very essentially facilitated, according to Strasburger, by the use of this carmine mixture, the color making the form of the nucleus come out most beautifully. The preparations should be examined in glycerine and mounted in that or in glycerine jelly.

6. *Beal's carmine solution.*[64] Put 0.6 g. of pulverized carmine in a test tube, pour over it 2.3 cc. of concentrated ammonia fluid and heat. After the solution is completed let it stand for an hour and pour the red fluid into a mixture which is made of 60 cc. of water, 47.5 cc. of concentrated glycerine,

[63] Frey, *l. c.*, p. 64. — Dippel, *l. c.*, Bd. I, p. 285. — Strasburger, Zellbild. u. Zelltheilung, 1880, p. 9.

[64] Frey, *l. c.*, p. 95. Poulsen, *l. c.*, p. 37, trans. p. 52.

and 19 cc. of absolute alcohol, stir up with a glass rod, let it stand for some time and then filter.

For the study of the protoplasmic contents of the cells in filamentous algæ (*Spirogyra*) one should, according to Strasburger,[65] lay the fronds in a 1 per cent solution of chromic acid, for at least four hours. Then repeatedly wash in distilled water and lay in a mixture of Beal's carmine and camphor, diluted with 8 parts water, 1 part glycerine and 1 part alcohol. There will follow after some time a rosy coloring of the protoplasmic cell contents which makes its fine structural relations very distinct.

7. *Grenacher's alum-carmine.*[66] The alum-carmine represents an exceptionally fine staining fluid for cell nuclei, which, according to Grenacher is prepared in the following way. Dissolve in 100 cc. of distilled water, 0.5 to 1 g. pulverized carmine and 1 to 5 g. potassium alum or common alum. Tangl[67] recommends a like composition which colors nuclei as well as cellulose membrane. "Dissolve alum in water to saturation, mix the solution now with any desired quantity of carmine, boil about ten minutes and filter after cooling." The staining requires from 5 to 10 minutes. The preparation keeps very well in glycerine, and the use of this stain gives very instructive specimens. In order to obtain clean specimens it is very much recommended to previously harden the part of the plant which is to be stained in absolute alcohol. This not only facilitates the imbibition of the stain but also has the further advantage that the staining capacity of the substances in the cells remains unchanged.

8. *The Schweigger-Seidel acid carmine solution.*[68] A common ammoniacal carmine solution is mixed with an excess of acetic acid and filtered. This tincture stains diffusely at first. Then put the colored preparation in glycerine to which has been added $\frac{1}{200}$ part of muriatic acid. This will remove the color from the cell body generally leaving the stain only in the nu-

[65] Strasburger, *l. c.*, p. 172.

Grenacher in Archiv für Mikrosk. Anatomie, Jahrg. 1879, p. 465, thence in Zeitschrift *f.*, Mikroskopie, Jahrg. II, 1879, p. 55.

[67] E. Tangl in Pringsheims' Jahrb., Bd. XII, 1880, p. 170.

[68] Frey, *l. c.*, p. 96.

cleus. In order to mount in glycerine one should wash the preparation in water containing acetic acid.

All carmine mixtures are used principally for staining nitrogenous, most of all protoplasmic, substances. The nucleus takes the stain with particular avidity, and mostly in greater quantity than the surrounding protoplasm. All these substances absorb carmine but never till after death. Cellulose (with the exception of some modifications), starch and other cell elements without nitrogenous contents, do not absorb the coloring matter of most of the carmine compounds.

38. Picro-carminate of Ammonia.

(Picro-Carmine.)

A rapidly staining medium is recommended by Treub for the investigation of nuclei and by Weigert for studying bacteria, and which was first introduced into zoö-microscopy by Ranvier, viz. picro-carminate of ammonia. According to Frey[69] it is thus made. To a concentrated aqueous solution of picric acid add to saturation, drop by drop, an ammoniacal carmine solution. Then evaporate to one-fifth the original volume. The cold solution deposits a small sediment of carmine. Then filter and evaporate to dryness when a red, ochre-yellow powder will be obtained. Dissolve portions of this in the preparation of the reagent 1 g. in 100 cc. water. Filtering from time to time is indispensable.

Baber (*l. c.*) mixes 1 g. of carmine in 4 cc. of concentrated ammonia, and 200 cc. of water, then adds 5 g. picric acid and shakes up and decants so that the undissolved excess of the picric acid remains behind. After the red liquid has stood for several days with frequent shaking, it is put in a shallow dish in the air to dry. The red powder should then be dissolved, 2 parts to 100 of water, and after some days filtered through two layers of paper. The fluid should now be yellowish red without smell of ammonia. A drop on white filter paper gives on drying a yellow, red-bordered fleck. A couple of drops of carbolic acid keeps the stain from decomposition.

[69] Frey, *l. c.*, p. 96.

Weigert's[70] formula is as follows. Pour 4 g. common ammonia over 2 g. of carmine and let it stand for twenty-four hours; now all that is soluble is dissolved. Then add the smallest possible quantity of acetic acid till the first faint traces of precipitation are seen. After standing another twenty-four hours add some ammonia, drop by drop. According to Baber the mounting fluid which is best for specimens stained with this reagent is one made with 10 drops glycerine, 10 drops water, and 1 drop of the reagent itself.[71]

39. Alcanna Tincture.

This reagent is recommended by N. J. C. Müller in an aqueous alcoholic solution as a test for resins and essential oils. A preparation of sufficient thinness, from some part of the plant that has little drops of resin in the cells, should be put on the slide and with it a clean fragment of alcanna, and to both add a drop of dilute alcohol. In a few minutes, two or three, the resin drops in the cell will be stained a lively red, and strangely enough, more intensely stained than the surrounding aqueous-alcoholic pigment solution. Protoplasmic masses without the resinous substances require from one-quarter to one-half hour in a like concentrated pigment solution before a distinct color is perceived. If the stained section is treated with alcohol and the colored drop disappears, no more treatment of the same drop with the same reagent will make the color perceptible to him. The following is the best process for preparations in water. Break from the alcanna a thin, even scale of perhaps about the size of the section to be tested. Rub it between the clean fingers with a drop of water to remove all attached powdered parts, and lay it on the section which is covered already with water. Then put on the cover-glass and at the edge of this a drop of alcohol. After two or three minutes remove the

[70] C. Weigert, Zur Tecknik der Mikroskop, Bacterienuntersuchung. Virchow's Archiv f. pathol. Anatomie, Bd. LXXXVIII, Heft 2, 8. Folge, Bd. II, Heft 2, 1881, pp. 275–315.

[71] Double stainings with this reagent and carmine, aniline, osmic acid, and picric acid have been frequently prepared. (*Cf.* Jour. Anat. and Phys., Vol. XV, 1881, pp. 349–354 Jour. Roy. Microscop. Society, 1881, p. 528, etc.)

fragment of alcanna, and with sufficient magnification you will find the drops in question stained a beautiful red. This will all happen as indicated if the alcanna is rich in coloring matter, but a very poor article is now often sold in the market. It is self-evident that if we apply the above mentioned test of washing out the stained section with alcohol we may easily distinguish between the drops of resin and essential oils, and those of fatty oils.[72]

[72] N. J. C. Müller, Untersuchung über die Vertheilung der Harze, etc., in Pflanzenkörper (Pringsheim's Jahrb., Bd. V, pp. 387–421).

CHAPTER V.

MICROSCOPICAL INVESTIGATION OF VEGETABLE SUBSTANCES.

For such a complete presentation of the microscopical investigation of the elementary substances of plants as is possible from the present standpoint of scientific microscopy, we must first of all begin by making such a classification of the very elaborate materials at hand as may be practically and scientifically justified. Since, as we have already remarked, a comprehensive presentation of this matter does not at the present time exist, we must here build from the very foundation. As we survey the rich amplitude of material which the investigations of the vegetable histologist and physiologist as well as those of the chemist have provided for us, it is not difficult to see, that the manifold substances which compose the organs of plants may be divided according to the frequency of their occurrence into two groups. First, we recognize a large series of plant substances which have a very wide distribution in the vegetable kingdom. We need refer here only to albuminous bodies which no plant lacks, or to cellulose, which, except in the very lowest plants, occurs likewise in all. So also starch, plant mucilage, sugars, chlorophyll combinations, etc., are widely distributed throughout the plant kingdom, and the instances in which they fail are comparatively very few. In contradistinction to these widely distributed bodies stands a series of substances whose occurrence is either limited to small groups of plants, or which are produced only by certain growths,— substances, at all events, which are of but secondary importance in the building up of plants. Belonging to this group, to mention some bodies of which microscopical analysis has already taken possession, are the coloring matter of algæ, tannic acid, resin, balsam, terpene, essential oils, coniferin, chrysophanic acid, and many others.

It is also not to be forgotten that many substances which are placed in the latter category have, perhaps, a much wider and more general distribution in nature than the present state of our science would justify us in assuming. Thus, for example, some physiologists have supposed that coniferin occurs in all woody growths. So also the investigations of Weinzierl (see p. 302) have shown that phloroglucin which hitherto had only been prepared synthetically by the chemist (benzole, on which for the 3 atoms of hydrogen are founded 3 groups of hydroxides of equal value, trioxhydro-benzol), or the so-called xylophilin of v. Höhnel (see p. 302), which, according to Weisner's investigations represents a mixture of phloroglucin and pyrocatechin, constantly occurs not only in woody but also in many herbaceous plants. But perhaps as a parallel to such statements, the objection may be raised that such a presentation as the one in hand must adapt itself exactly to the present state of our knowledge of these matters, and thence can be useful but for a limited period of time, and must necessarily become unserviceable with the further progress of science.

We now first consider those plant substances which are of universal distribution and of such we present the following. 1, Cellulose and its modifications; 2, Starch; 3, Dextrine; 4, Vegetable mucilage; 5, Gums; 6, Inulin; 7, Grape sugar; 8, Cane sugar; 9, Albuminous substances; 10, Chlorophyll; 11, The coloring matter of flowers; 12, Asparagin; and 13, Inorganic vegetable elements.

The question now lies before us, according to what points of view we *might* arrange these substances into a continuous series, and according to which of these points of view we should arrange them so as to be both practically and scientifically justified. Three points of view offer themselves to us. These substances may be grouped according to their morphological, physiological or chemical characteristics. In the first case we should have to place those things together which have a like or similar appearance: thus, in the first place, the materials forming the cell wall, then the solid, and finally the fluid and semi-fluid cell contents. We should then also distribute into one and the same group, starch and proteid grains, chlorophyll, calcium

crystals, etc., joining things which are altogether different. A classification of vegetable substances in accordance with their physiological functions is impracticable, simply on the ground that we know very little of what part many substances take in the vital processes, and of many others we know nothing at all. There remains to us, therefore, only the classification of these substances according to their chemical nature. And this is also, in fact, the most suitable, and the following chapter makes it its principal purpose to furnish directions for determining the chemical nature of the substances occurring in the interior of plants.

We make the classification upon the inorganic elements, and the absence or presence of nitrogen gives us the first characteristic for a wide classification. The first division, combinations containing no nitrogen, we designate collectively carbo-hydrates, and the second division nitrogenous combinations. The carbo-hydrates include the cellulose and the rest of those isomeric vegetable substances in which the formula $C_6 H_{10} O_5$ occurs. To the carbo-hydrates also belong the different kinds of grape sugars, $C_6 H_{12} O_6$, and the cane sugars, $C_{12} H_{22} O_{11}$. The nitrogenous combinations include the well-known albuminous substances (proteid bodies), vegetable coloring matter, of which chlorophyll may be designated as the most important, and finally asparagin as an amido-combination.

According to the point of view which we have here developed we get the following arrangement of those vegetable elements which have the widest distribution.

CARBO-HYDRATES.

Cellulose Group $C_6H_{10}O_5$

1. Cellulose.
2. Starch.
3. Dextrine.
4. Vegetable mucilage.
5. Gums.
6. Inulin.

Sugar Group.

7. Grape sugar $C_6 H_{12} O_6$
8. Cane sugar $C_{12}H_{22} O_{11}$

NITROGENOUS COMBINATIONS.

9. Albuminoids (proteid bodies).
10. Chlorophyll.
11. Coloring matter of flowers } Vegetable coloring matter.
12. Amido combinations, asparagin.
13. Inorganic vegetable elements.

It should be remarked in reference to this arrangement that the substances designated "matter" (Stoffe) are by no means such in the chemist's sense. A chlorophyll grain, for example, consists as is known of a large number of bodies which on their part again are not to be considered as simple chemical combinations. We should regard the present arrangement as nothing more than a grouping of important physiological individuals.

In the following analysis we shall first make ourselves acquainted in general with the qualities of each of the substances, and then describe the methods of microscopical reaction to be employed with each in order to recognize it with certainty. Very unimportant reactions, or those whose value has not yet been sufficiently established, will either not be mentioned, or will be but incidentally referred to, and on this account we once for all refer the reader to the "literature" to be found before each section.

A. SUBSTANCES OF UNIVERSAL DISTRIBUTION.

I. CELLULOSE AND ITS MODIFICATIONS.

Cellulose or cell-substance ($C_6 H_{10} O_5$ or $C_{12} H_{20} O_{10}$) presents in its pure state a solid, colorless transparent body. Cellulose appears in plants in the form of cell cuticle or cell wall. It is isomeric to the other members of the group of cell substances enumerated on p. 314, but in part varies much from them in its chemical and physical behavior. The isomerism explains the fact that many of these substances during the vital processes in the body of the plant can be easily changed into one another, as, for example, a transformation of starch into cellulose and of cellulose into gum often takes place.

Under the physiological conception of cellulose we are however to understand, in accordance with the science of to-day, not only pure cellulose, but also certain related substances, which result from the metamorphosis which cellulose undergoes in the life processes of the plant, and which in general are dis-

tinguished by containing more carbon and less oxygen than cellulose in the strict sense. These modifications some naturalists regard as different chemical individuals (Fremy), while the majority of chemists and physiologists see in them, as already mentioned, only modifications (Payen, Fromberg, Mulder). Thus Fremy specifies as chemical cellulose individuals of this sort, essential cellulose, paracellulose, vasculose, fiberose and cutose.[1]* If we can at present form no definite idea of the nature of the modifications of cellulose, it still appears probable, in opposition to the view of Fremy, and in accordance with the chemical investigations of Payen and later of Schultze,[2] as well also as in accordance with the studies of the botanists, that the change in the cellulose is produced by the molecular intercalation during the process of growth, of certain other substances into the cell-wall which originally consisted of pure cellulose. For example, we have the phenomenon of lignification, whereby the cellulose, as is well known, is transformed into lignin. This takes place by the intercalation of a substance, which Payen had already recognized and designated by the name of "incrusting substance," and which Schultze afterwards believed himself to have prepared pure.

The modifications of cellulose which have been distinguished with sufficient distinctness, are wood substances (lignin); middle lamella, intercellular substance, which very much resembles wood in many respects; cork substance (suberin) out of which is also composed the corky layer known as cuticle which regularly covers the epidermis (cutin), and the inclosing layer of the pollen grain (pollenin); finally, fungus cellulose. The latter to which one might suitably give the name of *fungin*, if this had not previously been used in another sense, was, till a short time ago looked upon by botanists of repute[3] as an

[1] Fremy, Comptes rend., t. XLVIII, p. 667, *ff.*, p. 862, *ff.*

* Fremy at present classifies the constituents of vegetable tissues under the following seven heads, the characters being derived from their chemical constitution. 1, Cellulose substances (cellulose, paracellulose, and metacellulose); 2, Vasculose; 3, Cutose; 4, Pectose; 5, Calcium pectate; 6, Nitrogenous substances; 7, Mineral elements (see at large, Ann. Sci. Nat. XIII, 1882, pp. 360–382, condensed in Jour. Roy. Micro. Soc., Vol. III, 1883, pp. 232–5). A. B. H.

[2] Schultze in Chem. Centralbl., 1857, p. 321.

[3] De Bary, Morphologie d. Pilze, Flechten u. Myxomyceten (Bd. II, von Hofmeister's Handb., p. 7, *ff.*).

isomeric substance to cellulose in the sense of Fremy. But according to the latest investigations of Richter,[4] it appears that this also is nothing else than common cellulose with foreign admixtures, mainly albuminous substances. On the contrary, it remains questionable if the medulin of the chemist forms a like sharply pronounced modification of cellulose. Omitting now these modifications, we have still to describe, under the true cellulose, those conditions which arise from the disorganization of cellulose and lead to isomeric combinations, as amyloid, plant mucilage, caoutchouc, arabin, bassorin, etc., and which we may suitably designate by the expression muculent cellulose.

Classified according to their characteristic qualities, cellulose and its related substances may be arranged in the following manner:

1. *Essential Cellulose*, cell substance, soluble in cuprammonia, concentrated sulphuric and chromic acid. It colors blue or violet with iodine and sulphuric acid, or with chlor-iodide of zinc. It has no admixture of foreign substances.

2. *Muculent Cellulose*, frequently soluble in cuprammonia as well as in concentrated sulphuric acid and chromic acid. It seldom colors blue with iodine and sulphuric acid, or with chlor-iodide of zinc, but mostly yellow or yellowish, or remains quite colorless. It is distinguished from all other forms of cellulose by its swelling.

3. *Wood Cellulose*, *Lignin*, insoluble in cuprammonia, soluble in concentrated sulphuric and chromic acid; is colored almost always yellow with iodine and sulphuric acid, or with chlor-iodide of zinc. With phloroglucin and hydrochloric acid rose red (distinguished from all other kinds of cellulose); possesses less oxygen than pure cellulose.

4. *Middle Lamella*, *Intercellular Substance*, insoluble in cuprammonia, insoluble in concentrated sulphuric and chromic acid, colors yellow with iodine and sulphuric acid, or with chlor-iodide of zinc.

[4] Richter in Sitsungsber. K. K. Acad. d. Wiss. Wien, Bd. LXXXIII, I Abth., 1881, p. 510.

5. *Cork Cellulose, Suberin* (including cutin, pollenin), insoluble in cuprammonia, insoluble in concentrated sulphuric acid and chromic acid (or in the latter very slightly soluble), colors very seldom yellow, mostly brown with iodine and sulphuric acid; gives cerinic acid reaction with Schultze's mixture. It contains an admixture of certain nitrogenous substances.

6. *Fungus Cellulose*, insoluble in cuprammonia, very slightly soluble in concentrated sulphuric acid. It very seldom takes a blue color with iodine and sulphuric acid, or with chlor-iodide of zinc. It occurs only in fungus (and lichens) and appears to contain an admixture of albuminous substances.

From these varieties of modified cellulose, may be obtained the pure cellulose which will give a true cellulose reaction with iodine and sulphuric acid, or chlor-iodide of zinc, if they, according to their nature, be treated with water, or with alcohol or ether, dilute acids, nitric acid, together with potassium chlorate, or caustic potash.

1. Cellulose in the narrow sense. (Cell substance.)

The most important reagents for testing cellulose are those iodine solutions particularly described on p. 285; in contradistinction to these the other methods for testing cellulose are of a very subordinate nature. They are commonly employed only when by the reactions of the iodine solutions, we are not able to determine with definiteness whether we have pure cellulose before us or one of the more closely related modifications of it. In this case one must observe its behavior when treated with mineral acids, alkalies, cuprammonia, alum carmine, copper sulphate and potassium, or in a secondary way by its negative behavior towards phenol-hydrochloric acid, aniline sulphate, phloroglucin and indol, which will demonstrate its distinction from other cellulose modifications.

A. Behavior of Cellulose to Iodine reagents.

Literature. J. B. Read, On the chemical composition of vegetable membrane and fiber (Lond. and Edinburgh, Phil. Magazine, Vol. XI, 1837, p. 421, *ff.*) Schleiden, Einige Bemerk. über die sogen. Holzfaser der Chemiker (Wiegmann's Archiv, Jahrg., IV, 1838, Bd. I, p. 49, *ff.*) —Schleiden, Einige Bemerk. über d. vegetabil. Faserstoff und sein Verhalten z. Stärkmehl (Poggendorff's Annalen, Bd. XLIII, 1838, p. 391). —Schleiden, Beiträge, etc., 13, 160, 164, 172, u. a. and O. —Schleiden, Noch einige Bemerk. über d. veget. Faserstoff, u. sein Verh. z. Stärkmehl (Flora, 1840, Bd. II, p. 737, *ff.*, p. 753, *ff.*)—Mohl, Einige Beobacht. über d. blaue Färbung d. veget. Zellmembran durch Jod (Flora, 1840, Bd. II, p. 609, *ff.*, p. 625, *ff.*)—Payen, Mém. Sur la Compos. chim. du tissu propre des végét. phanérog. (Ann. des sc. nat. 2e sér., t. XIV, 1840, pp. 73–100.)—Lantzius-Beninga, De evol. sporid. musc. Gött., 1840, p. 7.—Mohl, Verm. Schriften. Tübing., 1845, p. 337, *ff.*, u. daselbst a. v. a. O.—Mohl, Bildet d. Cellulose d. Grundlage sämmtl. veget. Membranen? (Bot. Zeitg., 1847, p. 497, *ff.*).—Mohl, Die veget. Zelle, p. 30, etc. (*cf.*, auch Wagner's Handwörterbuch, Bd. IV, p. 189, etc.). —Dippel, Beitr. z. Lösung der Frage, etc. (Bot. Zeitg., 1851, p. 409, *ff.*)—Schacht, D. Pflanzenzelle a. v. O., z. B. p. 143, *ff.*—Pringsheim, Algologische Mittheilungen (Flora, 1852, p. 470, *ff.*)—Hofmeister, Ueb. die zu Gallerte aufquell. Zellen der Aussenfläche v. Samen u. Perikarpien (Ber. Kön. Sächs. Gesellsch. der Wiss. Bd. X, 1858, p. 21, *ff.*)—Fremy, Rech. chim. sur la compos. des Cellules végét. (Comptes rendus, t. XLVIII, 1859, p. 202, *ff.*)—Fremy, Caractères distinctifs des fibres lign., des f. corticales et du tissu cellulaire qui consiste la moëlle des arbres (*l. c.*, t. XLVIII, 1859, p. 275, *ff*)—Payen, Différents états de la cellulose dans les pl. (*l. c.*, t. XLVIII, 1859, p. 772, *ff.*)—Mulder, Physiol. chem., p. 475. —Kabsch, Unters. üb. d. chem. Beschaffenh. d. Zellwände (Pringsheim's Jahrb., Bd. III, 1863, p. 357, *ff.*)—Nägeli, Ueb. d. Verhalten d. Zellhaut zum Jod. (Sitzungsber. d. bayer.

Acad. d. Wiss., 1863, Bd. I, p. 383, *ff.*)—Nägeli, Uber die Reactionen von Jod auf Stärkekörner u. Zellmembr. (*l. c.*, p. 483, *ff.*)—Hofmeister, Handb. d. physiol. Bot. Bd. I, p. 252, *ff.*, etc.—Sach's Handb. d. Experimentalphys. d. Pfl., p. 433, *ff.*—Hofmeister, D. Lehre v. d. Pflanzenzelle, Lpz., 1867, a. v. O.—Dippel, Mikroskop, Bd. II, p. 6, *ff.*—Sach's Lehrb. d. Bot. p. 19, *ff.*—Nägeli und Schwendener, Mikrosk., p. 474, p. 517, *ff.*, p. 549, etc.—Strasburger, Zellbild. u. Zellthiel. III. Aufl. 1880, a. v. O.—Poulsen, Botanisk Mikrokemi, p. 49, *ff.* (Trans. p. 75).—Richter, Beitr. z. genaueren Kenntniss der chem. Beschaffh. der Zellmembranen bei den Pilzen (Sitzungsber. K. K. Acad. d. Wiss. Wien, Bd. LXXXIII, 1 Abth., 1881, pp. 494–510).

When, as appears from the investigations of Strasburger,[5] in the dividing of a cell, the primary cell plates form between the connecting fibres of the separated nuclei, there will be introduced into these by intercalation very fine granules, which by their behavior towards iodine solutions (see below) will demonstrate themselves to be grains of starch of the utmost minuteness.[6] But as soon as the granules are transformed into the substance of the outer cell wall they will then give no reaction[7] whatever, either by the addition of chlor-iodide of zinc, or iodine and sulphuric acid. These primary cell plates are either transitory, that is to say, are absorbed and give place to cellulose plates or they coëxist with these and are employed in their formation.

The completed but very young cell membranes of the merestematic tissue are not often colored (Dippel), or if at all yellow (Solla)[8] by the use of iodine and sulphuric acid or chlor-iodide of zinc. If, however, they are previously treated with muriatic acid, or potash lye or have lain for a short time in water in which the process of fermentation is taking place they would be colored blue by a brief subjection to the influence of the iodine reagent.[9]

[5] Strasburger. Zellbild. und Zellth. III, Aufl., 1880, p. 1, *ff.*

[6] Strasburger, *l. c.*, p. 16, Table I, Fig. 6–9.

[7] Strasburger, *l. c.*, p. 13.

[8] Dippel, Mikrosk., Bd. II. p. 7, *f.*—Solla in Oesterr. Bot. Zeitschr. Jahrg., 1879, p. 351.

[9] Richter in Sitzungsber. der K. K. Acad. d. Wiss. Wien, Bd. LXXXIII, 1 Abth., 1881, p. 498.

The older cell layers, which consist of pure cellulose, are not colored at all, or only with a yellowish or brown-yellowish or reddish tint by the addition of freshly prepared iodine water.[10] But if the iodine water contain traces of hydriodic acid a blue or violet color will be produced (Nägeli). But if the preparation which has been impregnated with iodine water be treated to a drop of sulphuric acid or caustic potash, there immediately appears an intense blue color, while neither muriatic nor nitric acid will produce this staining (Meyer, Schleiden). Chlor-iodide of zinc solution colors pure cellulose blue under all circumstances and is the most important reagent for it. Zinc chloride causes the cell walls to swell very quickly and so disturbs their natural relations to each other. This can, however, be prevented for a considerable time by suitably diluting the fluid with water or potassium iodide of iodine solution. Chlor-iodide of zinc is almost always to be preferred to iodine water and sulphuric acid, since the sulphuric acid very quickly destroys the whole tissue.

The intensity but not the shade of the blue or violet color which is produced by the iodine solution is conditioned upon the quantity of the intercalated iodine in the membrane.

Nägeli[11] who subjected the behavior of pure cellulose membrane towards iodine to a very searching investigation reached the following principal results.

The quantity of the intercalated iodine determines in general not the character but only the intensity of the color. Each tint (yellow, orange, red, violet, blue) may be made bright by less iodine and intense by the use of a greater quantity. One may observe in single cases a transition from bright yellow to dark blue when during the reaction of the iodine hydriodic acid is formed. In other cases the absorption of more iodine changes the color from blue to brown when the membrane consists of

[10] The walls of the spore utricle of the lichens are an exception to this, however, as they are colored blue by the use of iodine water alone. (Nägeli in Sitzungsber. Bayer. Acad., 1863, Bd. I, p. 485, *ff.*). the blue color becomes, however, more intense by addition of sulphuric acid (Richter, *l. c.*, p. 496). For the rest see also Mohl in Flora. 1840. II Bd. p. 614, and G. Dickie in Annals of Nat. Hist., 1839, p. 165.—Concerning the reactions of the cellulose walls of spores of algæ, see Pringsheim in Flora, 1852, p. 470, *f.*

[11] Nägeli, concerning the reactions of iodine on starch grains and cell membranes (Sitzungsberichte der Bayerischen Acad. 1863, Bd. I, pp. 524, 530, 532, 535, 539, 541, 543).

a mixture of two different materials which are acted upon differently by the iodine.

Cell membranes which are permeated by water, and have received some one color by iodine, retain this color when the water is drawn out of it at the common temperature, and when otherwise no chemical or physical change has occurred. But, on the contrary, if some substance is dissolved by the interpenetrating water which is again concentrated by the evaporation, it may so effect the arrangement of the molecules of iodine as to cause a greater or less change of color.

Membranes colored by iodine, which may become uncolored either in the moist or dry condition, frequently change their color more or less. These transformations always proceed in the direction from blue through red to yellow.

When a cell membrane will not immediately stain by iodine and water it may be colored by the action at the same time, of hydriodic acid (which is formed by the prolonged action of iodine on different organic combinations as well as by drying them with iodine), or of potassium iodide, or ammonia iodide, zinc iodide, phosphoric acid or sulphuric acid, in other cases also by sulphuric acid after a more or less energetic treatment by caustic potash or nitric acid.

The treatment with hydriodic acid, potassium iodide, ammonium iodide, with sulphuric and phosphoric acid, caustic potash and nitric acid removes without doubt a less or greater quantity of foreign substances contained in the membrane which are soluble in that particular combination. This purifying of the cell membrane may in many cases facilitate the bluing, but it is in no case the only determining condition of it.

Treatment by the above named reagents causes a greater or less swelling of the membrane but this loosening up of the tissue is in no case the cause of the bluing.

For the bluing of the cell membrane with iodine and water (except in the case of the lichen tissue) there is required the presence at the same time of an assisting combination, hydriodic acid, potassium iodide, ammonium iodide, zinc iodide (or another metallic iodide), sulphuric acid, phosphoric acid, zinc chloride (?). But, perhaps, the sulphuric and phosphoric acid

do not act directly but indirectly by favoring the formation of hydriodic acid, through the decomposition of alcohol, or of organic combinations of the cell, so that thence the blue color is almost exclusively conditioned by the presence of a definite quantity of an iodine combination.

B. Behavior of Cellulose towards Mineral Acids.

Of these, concentrated sulphuric and chromic acid are particularly adapted to give the wished-for demonstration. In opposition to hydrochloric acid which leaves the cellulose almost perfectly unchanged, and to nitric acid which when cold causes only a swelling of the cell membrane and dissolves it only by boiling, the other acids named dissolve the cellulose at ordinary temperature and in a very short time. On the contrary the modifications of cellulose, middle lamella, suberin, together with cutin are not soluble in them (see below). However, it should be stated that (probably all) wood membranes share with cellulose in the narrow sense, its solubility in sulphuric and chromic acid. If a section through the stem of a plant be put into concentrated sulphuric acid all that part which consists of lignin and pure cellulose will be dissolved out, while suberized layers and cuticularized combinations, as well as the middle lamella of the woody tissue, will remain behind intact. Cellulose is changed by sulphuric acid into an isomeric body to which Schleiden gave the name amyloid, because while it in itself remains unchanged it effects changes in other bodies by contact, or "catalysis" as the chemists say. This amyloid stands very near to starch as we infer from the fact that by the application of iodine water it takes on a very intense blue color. On this circumstance rests the above many-times-mentioned reaction of iodine and sulphuric acid on cellulose, and is conducted in the following way. Put the section to be examined in freshly prepared iodine water for a short time. Take it out and remove as much as possible of the adhering fluid, lay it on a slide, put on a cover glass, put the preparation under the microscope, and add at the edge of the cover-glass a large drop of concentrated

sulphuric acid. Now look quickly in the microscope and see how all the true cellulose membranes take on an intense blue color, while all the modifications of cellulose, lignin, middle lamella, and suberin are stained yellow or brown. After a few moments, however, the blue reaction becomes indistinct because of the destruction of the tissue which goes rapidly forward.

C. Behavior of Cellulose towards Alkalies.

In comparison with the destructive effect of mineral acids upon cellulose, the alkalies take but little if any hold upon it. Ammonia itself even in a concentrated state, and when the section is boiled in it for a short time, does not alter the substance of the cell walls, while concentrated or nearly concentrated potash lye will only cause them to swell. By washing out the section treated with potash, and putting it in absolute alcohol the cell walls will resume their original form, on which account Hanstein's method of bleaching is recommended (see p. 199); compare also what is said concerning Russow's potassium alcohol.

D. Behavior of Cellulose towards Cuprammonia.

Schweizer[12] found in 1857 that cotton laid in cuprammonia very quickly dissolved and assumed a jelly-like consistency, thence changed into a mucilaginous fluid which on being diluted with water was filtered. On applying muriatic acid a precipitate was thrown down which was colored brown by potassium iodide and chlorine water, showing that the cellulose was not changed to starch by the process. Cellulose is distinguished from all its modifications by the characteristic of its being soluble in cuprammonia. Put a drop of the freshest possible preparation of the reagent upon a damp section lying under the cover-glass and then observe how the cellulose walls gradually swell up. Afterwards the outlines become indistinct and the pro-

[12] Schweizer, Jour. prakt. Chem., Bd. LXXII, p. 111—see also above, p. 244, *ff.*, then the treatise of Fremy and Payen cited on page 268.

cess ends with a perfect solution of the whole cellulose structure. It may be remarked in this connection that the modifications of cellulose may be dissolved in cuprammonia when the intercalated incrusting substance has previously been removed. This is most readily accomplished by Schultze's maceration process, with potassium chlorate and nitric acid (see p. 163). Boil the section with this mixture in a test tube, wash out the undestroyed portion with water and put it on the slide with a drop of cuprammonia solution.

E. Behavior of Cellulose towards Alum Carmine.

Literature. E. Tangl, Concerning the open communication between the cells of the endosperm of some seeds (Pringsheim's Jahrb., Bd. XII, 1879–81, p. 170, *ff.*)

According to Tangl, Grenacher's alum carmine (p. 308) offers a superior means of distinguishing pure cellulose membrane from that which has been cuticularized or changed to cork. For while the former eagerly takes up the coloring matter and after five to ten minutes becomes an intense red, the latter remains uncolored. The color keeps very well in glycerine, and gives us a very instructive specimen and particularly so in respect to the vascular bundles. In order to get a beautiful stain of the nucleus and plasma one should previously harden the preparation in absolute alcohol (see above p. 308).[13]

F. Behavior of Cellulose towards Potassium-Copper Sulphate.

Literature. Sachs, Concerning some new methods of microchemical reactions (Sitzungsberichte der K. acad. d. Wiss., Bd. XXXVI, 1859, p. 1–22). Sachs, Micro-chemical investigations (Flora, 1862, p. 288, *ff.*). Sachs, Concerning the substances which furnish the material for the growth of the cell wall (Pringsheim's Jahrb., Bd. III, 1863, p. 187, *ff.*).

[13] Tangl states, *l. c.*, p. 173, also that the cellulose membrane of cambium and older parenchyma cells absorbs the blue coloring matter from an aqueous decoction of logwood in insoluble modification if this contains an addition of sulphate of iron and is applied cold. However, the same thing may happen in modified cellulose membrane so that it will not serve as a test of cellulose.

Sachs has given a process of bluing membranes which contain a certain kind of cellulose by treatment with copper sulphate and potassium solution. The method is as follows. A very thin section, if possible thinner than the thickness of a layer of cells, should be laid for a long time in a concentrated solution of copper sulphate. It should remain in this from five to ten minutes, or several hours, or a day even, according to its nature. Then remove the section and lay it for a few minutes in water in order to wash off the salt solution. For this purpose it is necessary to put the section in a considerable quantity of water and not merely in a drop on the slide. The better way is to take the section in the forceps and move it back and forth several times in pure water. Then in a porcelain saucer which holds 8 or 10 cc. make a strong potassium solution of 1 part by weight of water, and 1 part caustic potash, and put the section in it for a short time, till in certain of the cell walls there appears a blue tint and in others a yellow color, while others still remain colorless. Lying in a drop of the fluid the section can then be examined. Then put the section in a small porcelain cup and boil it for a few minutes in the potash solution. This intensifies the color and makes it appear now for the first time general. If the color is too transparent one must take a section sufficiently thick to make it distinct and characteristic.

By this method many but not all cell walls consisting of cellulose will be colored an intense blue, while others will remain colorless. Those cell walls which are colored yellow with iodine are not cellulose in the strict sense and are colored yellow or orange yellow by this method. So, according to Sachs, the peripheral layer of parenchyma of the germ-root of the horse bean (*Vicia faba*) takes on a beautiful blue stain; also parts of the parenchyma, the very young vascular bundles, wood cells, and bast cells of the germ-root of *Phaseolus multiflorus*, the subepidermal layer from the blooming branch of the gourd, and generally all collenchyma cells, young bast cells and wood cambium become blue, while commonly the thick walled parenchyma cells remain uncolored. The walls of older bast cells color yellow or yellow orange, and all lignified elements of the

wood bodies.[14] Since a characteristic reaction will not take place in all cellulose walls by this method, its application therefore is not a sufficient test without verification by iodine reactions.

2. Mucilaginous Cellulose.

Literature. Meyen, Die Secretionsorgane der Pflanzen, Berlin, 1837, p. 36, etc. — Schleiden in Wiegmann's Archiv, 1838, p. 145.—Deciasne, Sur la structure des poils qui couvrent le péricarpe de certaines composées (Ann. des Soc. Nat. IIe, Sér. t. XII, 1839, pp. 251–254).—Meyen, Pflanzpathologie, Berlin, 1841, p. 235. — Schleiden, Beitr. z. Botanik (1844), p. 137. —Mohl, Einige Bemerk. über den Bau der vegetab. Zelle (Botan. Zeitung, 1844, p. 323, *ff.*).— Mohl, Vermischte Schr. a. a. O.—Kippist, On the existence of spiral cells in the seeds of Acanthaceæ (Trans. of the Linn. Soc. of London, 1845, Vol. XIX, pp. 65–76).— Kützing, Grundzüge der philosoph. Botanik, Leipzig, 1852, Bd. I, p. 195, *ff.* — Cramer, Botan. Beiträge, Zurich, 1855, p. 1, *ff.*— Unger, Anat. und Physiol. der Pflanzen, Pest, 1855, p. 78, 119, Taf. IV. — Karsten, Ueber die Entstehung des Harzes, Wachses, Gummis, und Schleimes durch die assimilirende Thätigkeit der Zellmembranen (Bot. Zeitg., 1857, p. 313, *ff.*).—Mohl, Ueber die Entstehungsweise des Traganthgummi (Bot. Zeitg., 1857, p. 33, *ff.*)— Hofmeister, Uber die zu Gallerte aufquell. Zellen der Aussenfläche von Samen und Perikarpien (Berichte der Sächs. Gesellschaft zu Leipzig, Bd. X, 1858, pp. 18–36).—Trécul, in Comptes rendus, 1860; Journal de l'Institut, 1862, p. 241.— Wigand, Ueber des Desorganisation der Pflanzenzelle, insbes. über die physiol. Bedeutung von Gummi und Harz (Pringsheim's Jahrb., Bd. III, 1863, pp. 155–182).— Frank, Uber die anat. Bedeut. und die Entstehung der veget. Schleime (Pringsheim's Jahrb., Bd. V, 1866, pp. 161–200).— Hofmeister, Handb. d. physiol. Botan., Bd. I (1867), p. 258, *ff.*—Hanstein, Ueber die organe der Harz- und Schleimabsonderung in den Laubknospen (Botan. Zeitg., 1868, p. 697, *ff.*)—Behrens, Unters. über den anat. Bau des Griffels und der Narbe, Gött., 1875, p.

[14] Sachs, in Sitzungsber. Wein, *l. c.*, p. 18, 19, Flora, 1862, p. 295.

28, *ff.*—Prillieux, Etude sur la formation de la gomme, etc. (Ann. des sc. nat. VIe sér, t. I, 1875, pp. 176–200).—Reinke, Beitr. zur Anat. der an Laubblättern, besond. an den Zähnen ders. vorkomm. Secretionsorgane (Pringsheim's Jahrb., Bd. X, 1875, pp. 119–178).—Capus, Anatomie du tissu conducteur (Ann. des sc. nat. VIe sér., t. VII, 1878.)—Behrens, Die Nectarien der Blüten (Flora, 1879, p. 118, *ff.*, 144, *ff.*, 233, *ff.*, 440, *ff.*).—Dalmer, Ueber die Leitung der Pollenschläuche bei den Angiospermen (Jenaische Zeitschr., *f.*, Naturwissenschaft, Bd. XIV, 1880, a. a. O.).

Meyen and Unger have already observed the fact that certain cells and cell groups whose walls originally consisted of true cellulose were gradually transformed by a process of degeneration into isomeric carbo-hydrate combinations which in their chemical but principally in their physical behavior are more or less different from cellulose. Although the finished product of the muciparous process cannot be any longer regarded as cellulose (it will be treated more in detail under the section "vegetable mucilage"), yet in the first stages of the transformation of cellulose to mucilage it is so much like cellulose that it should be described in this place. The cellulose walls of the muciparous cells and cell groups have quite generally the capacity of swelling to several times their original volume by an unusual power of imbibing water; thus often, the whole cell, and always the muciparous tissue, loses its original form and is changed into a quite thin jelly. If the muciparous cell walls are thickened and stratified as are those of the *Astragalus*[15] species whose medullary rays furnish the gum tragacanth, the stratification will gradually become indistinct till at last the resulting mucilaginous mass will appear to be very nearly structureless.[16] The whole of the cell wall or only its outermost layer may be drawn into sympathy with this process and it ends with its transformation into a jelly more or less soluble in water.[17] In other cases a middle portion of the wall, which is often strongly developed, is dissolved into a fluid amyloid (Collagen) as for example in epidermal cells where, by the absorption of water,

[15] Mohl, in Botan. Zeitung, 1857, p. 33, *ff.*
[16] Compare Prillieux in Ann. sc. Nat., VIe Sér., t. I, pl. V. fig. 1.
[17] Mohl, *l. c.*, p. 42, *f.*

they swell out and become a mucilaginous jelly raising up the cuticle into blisters which finally burst.[18] In such cases there remains commonly besides the cuticle a thin layer of cellulose not participating in the process of disorganization, which separates the mucilaginous complex from the interior of the cell; but it is always a part of the strongly thickened cell wall which swells up to become mucilage, the process itself being much diversified in details.

The swollen substance sometimes agrees with cellulose both in its anatomical behavior and its chemical reactions. The jelly under discussion is colored, for example, blue (Hofmeister) with iodine water and iodine alcohol in the seeds of *Salvia horminum* and *Teesdalia nudicaulis* or pale blue (seeds of *Linum usitatissimum*). Iodine in connection with sulphuric acid of a certain definite concentration for each individual case colors it blue. The swollen layer of the seeds of *Salvia horminum* blued by iodine becomes reddish[19] by the addition of dilute sulphuric acid. In other cases the muciparous cellulose tissue behaves quite differently towards iodine reagents, often not taking any color whatever from any kind of iodine combination (Collagen, Hanstein). Where, as in the formation of gum tragacanth, the muciparous cell walls originally showed color with chlor-iodide of zinc solution, this reaction becomes weaker in the mass as the muciparous process goes on till finally it altogether ceases (Mohl). Other mucilaginous substances take a yellow color with iodine and sulphuric acid and with chlor-iodide of zinc;[20] only rarely do the older stages of muculent complex give the reaction of cellulose, viz., bluing by the two well-known reagents.[21] So the reagents show with their different effects, that in the process under discussion we have detected all transition from true cellulose to true mucilaginous matter.

As the most successful method of investigating muciparous cells the following may be mentioned. According to the statements of Fenzl[22] sections through muciparous seeds are best made by

[18] Hanstein in Bot. Zeit., 1868, p. 697 *ff.*— Behrens in Flora, p. 118, *ff.*, p. 232, *ff.*
[19] Hofmeister in Ber. Sächs, Ges. Leipzig, Bd. X, p. 30.
[20] Frank in Pringsheim's Jahrb., Bd. V, pp. 163, 165, 167, etc.
[21] Kutzing, Grundz. d. philos. Bot., Bd. I, p. 195.—Frank, *l. c.*, pp. 168, 181.
[22] Fenzl in Denkschr. d. Weiner Acad. VIII, Erkl. zu. Taf. I.

embedding them in stearine; having cut the section wash in alcohol to remove any shavings of stearine. The embedding medium described on pp. 186–7, given by Koch, may be employed. The section should be first studied dry or better still in alcohol, sometimes also in essential oils, because the use of water causes a rapid swelling. Since very delicate mucilaginous substances refract the light quite like alcohol and therefore can with difficulty be recognized in it, a small quantity of coloring matter, which will not be absorbed by the mucilage, should be added to the alcohol[23] in such cases. For this purpose nothing is better than the aniline dyes. If, after having studied the preparation in alcohol, water is added, the muciparous cells rapidly expand in a radial direction, at the same time the mucilaginous complex begins to swell very quickly, the swollen substances immediately separate in the water and directly disappear from observation. The addition of potassium solution will produce a swelling in the dry preparation.

3. Wood Substance. (Lignin.)

Wood or lignin ($C_{19}H_{24}O_{10}$)[24] forms the walls of all those cells which are changed into wood.[25] Lignified cells are commonly found in wood bodies; other lignified cells are, however, found isolated in the parenchyma tissue, as for example, the stone-cells which occur in the pith of numerous woody plants; cells of like name in the pulp of the fruit of the *Pomaceæ* and similar cell tissue in the bark layer, etc.

In all lignified cell walls, as mentioned on p. 316, there is intercalated an incrusting substance, which contains relatively more hydrogen and carbon than does cellulose in the strict sense. According to the later investigations of Singer and the earlier ones of v. Höhnel there are four substances which constantly

[23] Hofmeister, *l. c.*, p. 21.

[24] Particulars in Burgerstein in proceedings, d. K. Acad. Wein, Bd. LXX, 1 Abth., 1874, p. 338, Anm.

[25] For general lists of literature see, Sachsse, Chemie und Physiol. der Farbstoffe, Carbo-hydrates u. Proteinsubst. Lpz., 1887, p. 144, *ff.*—Niggl, Ueber die Verholzung d. Zellmembranen (Jahresber. Pollichia, 1881).—Ebermeyer, Physiol. Chem. der Pflanzen, Berlin, 1882, Bd. I, p. 174, *ff.*—Singer, Beiträge zur näheren Kenntniss d. Holzsubstanz, u. d. verholzt. Gewebe (Sitzungsber. d. K. Acad. Wien, Bd. LXXXV, 1882, 1 Abth., p. 345, *ff.*)

accompany lignified tissues, namely, Vanillin, Coniferin and a kind of gum which stands near to Arabin and perhaps represents a modification of the wood gum of Thompson, and finally a body which takes a yellow color with muriatic acid but whose chemical nature is still quite unknown. All these elements may be extracted by boiling the wood in water for a longer or shorter time; they are that which gives the characteristic reactions of wood-substances. In what relation they stand to lignin cannot yet be made out, but they indicate that that which we call lignin represents a mixture of several chemical individuals.

As the most important reagents for lignified cell walls, iodine solutions, aniline sulphate, phloroglucin, indol, and phenol-hydrochloric acid should be named.

As a distinction from pure cellulose, lignin does not dissolve in cuprammonia. It is, on the contrary, soluble in potash lye (more easily than cellulose) concentrated[26] nitric acid, sulphuric and chromic acid. Concentrated sulphuric acid blackens it in dissolving. Schulze's maceration mixture dissolves lignin very easily.[27]

A. Behavior of Lignin towards Iodine Reagents.

Literature. See the treatises cited on p. 319. Also Mohl, Einige Bemerk. über die Bau, der vegetab. Zelle (Botan. Zeitg., 1844, p. 307, *ff.*).—Schacht, Lehrb. d. Anat., etc., Bd. I, p. 16, etc.—Fremy, Recherches sur la comp. chim. du bois (Comptes rendus de Paris, t. XLVIII, 1859, p. 862, *ff.*)—Payen, Compos, de l'enveloppe des pl. et des tissus ligneux (Comptes rendus de Paris, t. XLVIII, 1859, p. 893, *ff.*)—Sanio, Einige Bemerk. über d. Bau des Holzes (Botan. Zeitg. 1860, p. 193, *ff.*)—Sanio, Vergl. Unters. über die Elementarorgane d. Holzkörpers (Botan. Zeitg., 1863, p. 85, *ff.*)—Sanio,

[26] Weak acids frequently make the layers of the cell wall come out very distinctly. Dilute sulphuric acid colors the young layers of lignified membranes a beautiful rose red (Hartig, Bot. Zeit., 1855, p. 213).

[27] According to Sanio (Bot. Zeig., 1860, p. 204), the wood substance is disintegrated into a granular mass which finally comes into the cell cavity, and if potash be now added this granular mass will be dissolved in it with yellow color.

Vergl. Unters. über die Zusammensetz. des Holzkörpers (Botan. Zeitg., 1863, p. 358, *ff.*—Mulder, Physiol. Chem., Bd. I, p. 209.—Dippel, D. Mikroskop, Bd. II, p. 96, *ff.*—Sanio, Zur Anatomie der gem. Kiefer (Pringsheim's Jahrb., Bd. IX, p. 65, *ff.*).—Sachs, Lehrbuch d. Botanik, p. 35.

Lignified cell-walls are colored yellowish, yellow or brownish by the application of any of the iodine reagents, potassium iodide of iodine, chlor-iodide of zinc, and iodine and sulphuric acid. The last-named shade is produced only by iodine and sulphuric acid, or very rarely by chlor-iodide of zinc; in this case, however, it shows only a brownish-yellow. The brighter and darker layers which are commonly perceptible in lignified walls either show no difference on the application of iodine and sulphuric acid, or else a distinction is apparent in the alternating stronger and weaker yellow color.

Pure yellow is seen in the perfectly lignified portion of the wall, while such parts as have been imperfectly lignified are shown by the mixed colors. They are either a transition between yellow and blue (blue-green or yellow-green) or reddish. The yellow color, produced by iodine reagents, which indicates lignification, shows itself through the whole extent of the cell-wall, or the wall will be but partially colored yellow. In this case it is commonly the peripheral layer of the wall which has the highest degree of lignification, while the inner portion next to the cell space is often less lignified.

The layer of the lignified cells which lies naked about the cell-cavity, and which is commonly an optically distinguishable thickening layer, called by Sachs "an inner shell," and by Dippel a tertiary membrane, behaves very differently. This is colored yellow by the use of iodine and sulphuric acid or chlor-iodide of zinc quite infrequently (Sanio)[28], but also reddish yellow, commonly violet, bluish or quite blue, consisting in these cases of slightly lignified or quite unlignified cellulose. Thus, for example, the innermost thickening layer of the wood cells of *Pinus sylvestris* consists of pure cellulose.

The incrusting substance may be removed from all lignified membranes by maceration, so that the cellulose which is left

[28] Sanio in Botan. Zeit., 1860, p. 202.

will give the characteristic reaction with iodine reagents (see p. 317 and p. 319, *ff.*).

Method. Prepare the thinnest possible section of the wood-tissues to be examined, and impregnate it if one is to produce the reaction with iodine and sulphuric acid (see p. 323) with potassium iodide of iodine or alcohol iodine, by laying it in a dish filled with these solutions for a longer or shorter time. The adhering solution should be washed off with distilled water, and the section laid upon the slide and a cover-glass put over; then a drop of concentrated sulphuric acid added and the reaction is quickly observed. In most cases this reaction is preferable to that by chlor-iodide of zinc. In the use of the latter reagent the moist section must sometimes remain in the solution several hours before the wished-for reaction will take place.

B. Behavior of Lignin towards Aniline Sulphate.

Literature. Runge, in Poggendorf's Ann., Bd. XXXI, 1834, p. 65.—Schapringer in Wochenschr. d. niederösterr. Gewerbevereins, Bd. XXVI, p. 326.—Wiesner in Karsten's Botan. Unters., 1866, Bd. I, p. 120.—Wiesner in Sitzungsber. der K. Acad. Wien, Bd. LXII, 1. Abth., 1870, p. 202 (Separatabdr., p. 32).—Wiesner, Die Rohstoffe des Pflanzenreiches.—Burgerstein, Unters. über d. Vorkommen u. die Entsteh. des Holzstoffes in den Geweben d. Pfl. (Sitzungsber. d. K. Acad. Wien, Bd. LXX, 1. Abth., 1874, pp. 338-355).—Höhnel, Ueber Kork. und verkorkte Gewebe überhaupt (*id.*, Bd. LXXVI, 1. Abth., 1877, p. 527).—Höhnel, Histochem. Unters. über d. Xylophilin u. d. Coniferin (*id.*, p. 663, *ff.*, u. a. a. O.).—Sachs, Ein Beitr. z. Kenntniss des aufsteigenden Saftstromes in transpirirenden Pflanzen (Arb. d. Botan. Institutes zu Wurzburg, Bd. II, Hft. 1, 1878, p. 150, *ff.*)—Gaunersdorfer, Beiträge z. Kenntniss der Eigenschaften und Entstehung des Kernholzes (Sitzungsber. d. K. Acad. Wien, Bd. LXXXV, 1882, 1. Abth., pp. 9–41).—Singer, Beiträge z. näheren Kenntniss der Holzsubstanz und der verholzten Gewebe (*id.*, pp. 345–360).

It was formerly supposed that chlor-iodide of zinc and iodine with sulphuric acid would always produce a yellow color in lignified cell-walls; but it was afterwards found out that though this was generally the case there were a few exceptions to the rule. Wiesner[29] was the first to point out that aniline sulphate was a reagent which in an acid solution (see p. 301) could be used as a test of wood substance in every kind of vegetable tissue. Already Runge and Schapringer had microscopically demonstrated that wood treated with this substance assumed an intense yellow color.

Later Burgerstein subjected the action of aniline sulphate upon lignin to a very exact investigation and found Wiesner's views confirmed in all points. That aniline sulphate (Wiesner's reagent) is a positive reagent upon lignin follows from this, that wherever this substance is chemically traceable in any tissue it shows the yellow color, but in all tissue from which the lignin has been withdrawn, by powerful oxidizing agencies such as chromic acid and Schultze's mixture, the reagent leaves no color.[30]

Method.[31] Put the tissue to be tested in a drop of distilled water and let a drop of the concentrated solution flow in from the edge. In tissue full of sap the reagent may be used without water. Potassium, sodium, and ammonia destroy the yellow color, but acid restores it again. The color is a pure gold yellow, yet the shade will depend upon the quantity used.

Burgerstein has investigated the different systems of tissue and their lignification, by means of aniline sulphate, and has arrived thereby at the following principal results.

Among thalophytes, only certain lichen tissue shows a slight lignification. The tissue of algæ and fungi is never lignified. In the vascular plants all the tissue systems are partially lignified (epidermal tissue, tissue of the vascular bundle, and fundamental tissue).

[29] Wiesner in Karsten's Botan. Unters., Bd. I, p. 120.

[30] Vesque (Comptes rendus de Paris, t. LXX:, p. 498) criticises the aniline sulphate, since it colors other membranes not lignified yellow. (??) Höhnel came to the contrary conclusion (Sitzungsber. d. K. Acad. Wien, Bd. LXXVI, 1 Abth., p. 528.)

[31] Burgerstein in Sitzungsber. der K. Acad. Wien, Bd. LXX, 1 Abth., p. 349.

A. Epidermal Tissue.[32] Epidermis, according to Schacht and Dippel, is never lignified. Burgerstein confirms this. He found this tissue lignified only in the seed wings of *Pinus* and *Abies*. The cuticle as well as the membranes of the stomata cells are never lignified. Hairs sometimes are and sometimes not. The collenchyma tissue which supports the epidermis is never lignified (Dippel asserts the contrary).[33]

B. Tissue of the Vascular Bundles.[34] The vessels in the xylem are, with few exceptions, always lignified (slightly so in the submerged parts of water plants and in very sappy land plants). Wood-cells are always lignified, both in the thickening cell-walls and in the middle lamella, as all naturalists admit.[35] The tertiary membrane, "innermost shell," is, according to Sanio,[36] usually lignified, but according to Sachs, Schacht and Dippel it is not. Burgerstein agrees with Sanio. The wood parenchyma is as Sanio[37] has already shown always lignified. The bast cells are, according to Sachs and Schacht, sometimes lignified and sometimes not. Burgerstein distinguished: *a*, bast cells lignified uniformly in all the layers of the membranes with the exception of the middle lamella, which appears always to be the most lignified (fully lignified bast cells); *b*, bast cells in which the primary and older secondary layers are becoming lignified, while the younger secondary and tertiary layers remain unlignified (partially lignified bast cells); *c*, bast layers whose whole substance is unlignified. The lignified bast cells are of most frequent occurrence. The sieve tubes are not lignified. The vascular bundle layer is always more or less lignified.

C. Fundamental Tissue.[38] The pith cells are for the most part lignified, especially those lying next the vascular bundles, likewise the cells of the medullary rays. The parenchymatous fundamental tissue is mostly not lignified, the leaf parenchyma never. Sclerenchyma cells are always lignified.

[32] Burgerstein, *l. c.*, p. 344, *ff.*
[33] Dippel, Mikroskop., Bd. II, p. 155.
[34] Burgerstein, *l. c.*, p. 346, *ff.*
[35] Sanio in Pringsheim's Jahrb., Bd. IX, pp. 50–126.
[36] Sanio in Prings. Jahrb., *l. c.*, Bot. Zeit., 1860, p. 202.
[37] Sanio in Bot. Zeitg., 1863, p. 98.
[38] Burgenstein, *l. c.*, p. 350, *ff.*

The lignifying process begins very early and advances very rapi lly forward. First the vessels lignify, then the wood cells, and the wood parenchyma, very soon thereafter the bast cells, and relatively later lignification begins in the pith.

C. Behavior of Lignin towards Phloroglucin.

Literature. Höhnel, Histochem. Unters, über d. Xylophilin u. d. Coniferin. I, Ueber d. Xylophilin (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXVI, 1. Abth., 1877, pp. 663–698).—Höhnel, Ueber den Kork, etc. (*id.*, p. 528).—Wiesner, Note über das Verhalten des Phloroglucins und einiger verwandter Körper auf verholzte Zellmembranen (*id.*, Bd. LXXVII, 1. Abth., 1878, pp. 60–66).—Singer, Beiträge z. näheren Kenntniss der Holzsubstanz und der verholzten Gewebe (*id.*, Bd. LXXXV, 1. Abth., 1882, pp. 345–360).—Poulsen, *l. c.*, p. 34 (Translation, p. 46, f.).[39]

This reagent was discovered by Wiesner. In an aqueous or alcoholic solution of even no more than 1 per cent, with the addition of muriatic acid, it stains lignin an intense red violet color.

Method. Put the section to be examined under a cover-glass and add a drop of the aqueous or alcoholic reagent according to circumstances, and no color will be produced. Then at the edge of the cover-glass put a drop of concentrated or somewhat dilute muriatic acid, and directly a very delicate violet color will begin to enter the lignified tissue, which becomes more and more intense till the whole shows a uniform, beautiful violet red color. By reversing the process and adding the acid first and the reagent afterwards the result is the same; the muriatic acid does not color the tissue noticeably. Vary the experiment by putting a drop of the reagent on a moist section and evaporating it almost all away and then adding the acid and the reaction takes place almost instantaneously.

According to v. Höhnel we operate with the extract of cherry

[39] References to the literature belonging to this subject may be found in the works of some of the older writers. There is a list of the related literature in v. Höhnel, *l. c.*, Bd. LXXVI, 1 Abth., pp. 693–698, besides which see Weiss und Wiesner, *id.*, Bd. XL, p. 276.

wood mentioned on p. 303, in a similar manner. Add to the fresh section a little quantity of the fluid letting it mostly evaporate and then add the acid. v. Höhnel found that in a transverse section of the stem of the *Anthericum liliago*, by this treatment the epidermis and the soft parenchyma lying directly beneath the young pith and the soft bast cells remained perfectly colorless. The vessels and the middle lamella of the woody tissue became dark violet; the more imperfectly lignified thickening layers of the wood cells and the elements of the sclerenchyma sheath bright violet.[40]

A transverse section through the stem of *Rumex obtusifolius* treated with an alcoholic solution of phloroglucin and concentrated muriatic acid showed the following. The epidermis and the strongly developed collenchyma layer lying beneath as well as the next following thin bark parenchyma remained perfectly colorless. In the vascular bundles all the woody parts inclusive of the less numerous wide vessels were colored a dark red violet. At the beginning of the reaction the color began to become distinct in the middle lamella first; afterwards it extended itself uniformly through the whole of the lignified walls. The outer layers of the pith are likewise strongly lignified corresponding to their red violet coloring. Towards the center of the section the pith cells become gradually a brighter violet and at the center remained quite colorless since they are not in the least lignified.

If the above described section were put in distilled water the violet red color would be changed to a brick red which would grow paler by degrees till at last the section would be quite colorless. It behaves the same way in alcohol or ether. The water dissolves the phloroglucin because the latter forms no chemical combination with the wood substance, it being only mechanically absorbed and intercalated in the cell walls. A section was carefully washed in water for a long time (five hours) and in spite of that took a faint violet color when muriatic acid was added, showing that there still remained a small quantity of the phloroglucin to produce the reaction. But if one removes the adhering acid from the section by passing it through

[40] V. Höhnel, *l. c.*, Bd. LXXXI, 1 Abth., p. 686.

water and then adds ammonia the color will change instantly to yellow and cloudy orange; the parts which before were tinted the intense violet will now show the deep shade of yellow. If now the alkali be washed out and acid again applied the violet color will be restored and of the same intensity as before. Ammonia (sodium or potassium lye, a basic salt) produces the decolorization of the phloroglucin stain. Muriatic acid (sulphuric acid, nitric acid, acid salts) restores the coloring again (see also v. Höhnel, *l. c.*).

D. Behavior of Lignin towards Indol.

Literature. Niggl, Das Indol ein Reagenz auf verholzte Zellmembranen. Mikrochemische Untersuch. (Flora, 1881, pp. 545–559, 561–566; also separate as a dissertation. Regensburg, 1881, 22 pages).—Singer, Beiträge zur näheren Kenntniss der Holzsubstanz und der verholzten Gewebe (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXXV, 1 Abth., 1882, pp. 346–360).

Indol, according to the recently published investigations of Niggl, produces a stain quite like that of the phloroglucin reaction.[41] By means of an acid it colors lignified membrane from a cherry red to red violet.

Method. The section to be examined is put on the slide with a drop of the aqueous solution of indol and a cover-glass laid over it. Then by means of a piece of blotting paper draw out a part of the solution and let flow in 1 to 2 parts of the dilute sulphuric acid mentioned on p. 304, whereupon the reaction immediately takes place. The specimen thus prepared keeps its beautiful color a long time. If concentrated acid be used, or the superfluous quantity be not drawn off, the color of the lignified membranes in a few weeks will be changed to brown-red. To prevent this let the acid work for an hour or two and then draw it out with filter paper and replace with glycerine.

Not only does the phloroglucin and indol reactions show the

[41] Still several other substances have been proposed for the same purpose in recent times, as Pyrol (Niggl), Orcin (Lippmann), Resorcin (Molisch, Wiesner), Pyrogalin (Wiesner), Hydrochinon (Niggl). I have not tested the working of all these substances, on account of the scarcity of some of them. On the contrary, I have carefully followed out Niggl's statements regarding indol and find them to be correct in all cases.

greatest agreement in respect to coloring, but also as I have found in the behavior of the colored membranes towards bases and acids. Wash out the acid from the indol-stained section and substitute ammonia and the violet color will disappear and in its place will appear a yellow to ochre colored tint. If this again be washed out and sulphuric acid be added the violet color will be restored. To distinguish it from phloroglucin it is to be mentioned that the indol stain does not, like that of phloroglucin, disappear by prolonged treatment with water. Sections stained with indol retain their color with undiminished intensity when they have lain in water for twenty-four hours. This makes the indol reaction preferable to that of phloroglucin.

Just as Burgerstein tested the effect of aniline sulphate on the different systems of tissue, Niggl has studied that of indol. We give in the following a brief résumé of his results. For the purpose of a more easy comparison with those of Burgerstein on p. 335 they are presented in the same consecutive order.

Thallophytes.

In algæ there appears to be no lignification except in the stout, warty, thickened membranes of some *Cosmarium* species. In the greater part of the fungi no kind of coloring appears. The exceptions are *Polyporus fomentarius* (a glimmer of red), *Ochrolechia pallescens* and *Trametes suaveolens* (distinct red). The thallus of the lichens behaves variously.[42]

Vascular Plants.

A. Epidermal Tissues. The epidermis is not colored by indol and sulphuric acid. The exception to this is the epidermal cells of the leaves of *Cinnamomum Culilawan*, *Cycas revoluta* and *flexuosa*, and of the needles of several *Coniferæ*. The cuticle is generally not lignified. The young sprouts of *Æsculus hippocastanum*, *Acer pseudoplatanus* and *Hippuris vulgaris* are an exception to this. The cuticle consists of numerous scales, and these often show a difference of behavior, the inner ones being sometimes reddened by indol. The mem-

[42] Niggl, *l. c.*, separatbdr., p. 5, *f.*

brane of the hairs is as frequently lignified as not. The stomata cells of the *Coniferæ* and the *Cycadeæ* are frequently colored red by indol. Collenchyma tissue is not lignified. The collenchyma of the stem and leaves of *Sapindus laurifolius* is an exception to this rule.

B. Tissue of the Vascular Bundles. The vessels are always lignified, the wood cells also, middle lamella and thickening layer always; tertiary membrane (inner shell) remains uncolored in *Astragalus*, *Caragana*, *Robinia* and *Cytisus*. The cells of the wood parenchyma are always lignified and indeed all three layers; in its younger state the innermost is not fully colored. The bast cells show considerable variation. Niggl observed, as did Burgerstein, bast cells perfectly lignified and others totally lacking in that; frequently, however, they were partially lignified. The outer layer, especially in the younger stages, is commonly reddened, while the inner one remains uncolored. Later, the middle part shows the reaction on the lignin. The sieve tubes are not lignified. The sheath of the vascular bundles is always at least partially lignified.[43]

C. Fundamental Tissue. Pith cells are commonly lignified; the cells of the medullary rays always, except in *Aristolochia sipho*. The hypoderm is sometimes lignified, but rarely the leaf parenchyma (*Cycas revoluta*, *C. flexuosa*). In the sclerenchyma cells the lignification can always be demonstrated.

The lignifying process begins earliest in the vessels.

E. Behavior of Lignin towards Phenol-muriatic Acid.

Literature. Tiemann und Haarmann, Ueber d. Coniferin seine Unwandlung in das aromat. Princip der Vanille (Ber. Deutsch. Chem. Gesellsch., Bd. VII, 1874, p. 608, *ff.*)—Tangl, Vorläuf. Mitth. über die Verbreitung des Coniferin (Flora, 1874, p. 239, *ff.*).—Rud. Müller, Ueber Coniferin (*l. c.*, p. 399).—v. Höhnel, Ueber den Kork und verkorkte Gewebe überhaupt (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXVII, 1 Abth., 1877, p. 700, *ff.*).—v. Höhnel, Histochem.

[43] Particulars in Niggl, *l. c.*, pp. 12–14.

Unters. über d. Xylophilin u. d. Coniferin. II, Ueber d. Coniferin (*id.* p. 699, *ff.*).—Singer, Beiträg. z. näheren Kenntniss d. Holzsubstanz und verholzten Gewebe (*id.* Bd. LXXXV, 1 Abth., 1882, p. 347, *ff.*)

It has long been known to the chemist that a pine shaving passed through carbolic and muriatic acid becomes blue. Afterwards it was proved by Tiemann and Haarmann that this coloring depended upon a substance existing in wood discovered by Th. Hartig and called coniferin. Tangl showed about the same time that a like reaction took place not only in coniferous wood, but also in *Sambucus nigra*, *Populus balsamifera*, *Fraxinus excelsior* and *Vitis vinifera*. v. Höhnel afterwards advanced the hypothesis that coniferin is an element of all wood tissue and that therefore the carbolo-muriatic reaction would serve as a test for wood tissue in general. Singer confirmed the views of v. Höhnel in a recently published investigation. While it was at first supposed that the reaction took place by first moistening with carbolic acid and then with muriatic acid, v. Höhnel first observed that the action of direct sunlight was also necessary.

Method (v. Höhnel).[44] Use the phenol-muriatic acid described on p. 302. With the perfectly clear solution we may obtain very clean and beautiful preparations. The section should not be too thin, and being moistened the least possible with the reagent it should be put under a cover-glass and set in the direct sunlight. An exposure of from one-half to one minute will be sufficient. A short time after this the section will have an intense color. If the exposure is for a longer time the strength and vividness of the color slowly fade and it becomes a sea-green or a yellow-green. The beautiful green color is characteristic of the really lignified membranes. All cells which would be colored blue with chlor-iodide of zinc, the epidermis, bark which is destitute of wood and cellulose, remain uncolored or colored yellowish by the muriatic acid in the reagent.[45] The section must be immediately examined because

[44] V. Höhnel, *l. c.*, Bd. LXXVI, p. 700, *ff.*

[45] Muriatic acid colors all wood tissues and also some others a more or less intense yellow. The color is, however, mostly very weak, the addition of water readily destroying it.

the green color is not durable. In lack of direct sunlight, concentrated artificial light may be substituted, though with poorer results. Very beautiful preparations are made from the aërial roots of orchids, stems of monocotyledonous plants, woods of *Evonymus*, *Æsculus* and the *Coniferæ*.

According to Tommaso and Donato Tommasi[46] the "coniferin reagent" is more distinctly effective if the section to be treated is first moistened with a mixture of carbolic acid and potassium chlorate, and then with the muriatic acid. By this means the blue stain comes out in diffused light, more rapidly and more intensely, and the preparation will not lose its color in a day.

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* *

For the purpose of determining the relative sensitiveness of lignin reactions, Singer[47] has experimented with solutions of like concentration of phloroglucin, indol, pyrol, aniline sulphate, resorcin, paratoluidin, pyrogalic acid, etc., by observing the effect of each upon tissue uniformly lignified, and has arrived at these results: "That with a 1 per cent solution of phloroglucin, indol and pyrol, we are able to produce vivid colors, quite uniform in their intensity. But also much weaker dilutions of the last named reagents carried were able to produce coloring, and in a 0.001 per cent concentration the limit of the effectiveness of phloroglucin was reached (see also p. 303), while the indol reduced to a 0.0007 per cent dilution colored coniferous wood, particularly after several hours. Pyrol is effective only when used in a stronger dilution.

Indol is thus the most sensitive reagent which we possess for testing lignification. But it does not prove to be the most useful; for, not to mention its great costliness (1 g. costing 70 M.) [in America $45], it will not keep well and requires the greatest caution in working with sulphuric acid which in a concentrated form destroys all vegetable tissue.

In consideration of this and in respect to the fact that pyrol is very difficult to make and changes its nature after a few

[46] Tommaso and Donato Tommasi, Ueber d. Fichtenholzreaction zur Entdeckung des Phenols im Urin. (Ber. Deutsch. chem. Gesellsch., 1881, p. 1834, *ff.*) — *Cf.* auch Singer, *l. c.*, p. 353, *ff.*

[47] Singer, *l. c.*, p. 358.

hours, we must give the preference to phloroglucin in combination with muriatic acid over all other lignin reagents."

After my experiments with aniline sulphate, phloroglucin and indol, I have to add to this that I agree with Singer in respect to the sensitiveness of indol; but that it appears to me as if the indol deserves the preference over the phloroglucin. Preparations stained with it seem to keep far better than when treated with phloroglucin, if one will carefully wash out the acid with distilled water and preserve in glycerine. The use of sulphuric acid of a dilution of one to four sufficiently insures the specimen against harm. And, finally, in respect to the decomposition of indol I must remark that I have an aqueous solution which now for more than seven months has perfectly preserved its efficiency and also its pungent smell.

4. Middle Lamella, Intercellular Substance.

Literature. Mohl, Verm. Schr., p. 314 ff., etc.—Mohl, Die veget. Zelle, p. 196.—Wigand, Intercellularsubstanz und Cuticula, Brschwg., 1850. Schacht, Lehrb. d. Anat. u. Phys. d. Gew., 1856, Bd. I, p. 108.—Sanio, Ueber Intercell. im Holz (Bot. Zeitg., 1860, p. 208–213).—Vogl, Ueber d. Intercellulars. u. die Milchsaftgef. in d. Wurzel des gem. Löwenzahns (Sitzungsber. der K. Acad. d. Wiss. Wien, Bd. XLVIII, 2 Abth., 1863, pp. 668–690)—Wiesner, Unters. über d. Auftreten v. Pectinkörpern in den Geweben d. Runkelrübe (*id.* Bd. L, 2 Abth., 1865, pp. 442–453)—Hofmeister, Lehre v. d. Pflanzenzelle, 1867, sec. 31.—Wiesner, Einl. in d. techn. Mikroskopie, Wien, 1867, pp. 62, 244, 246, etc.—Dippel, Die Intercellulars. und deren Entstehung. Rotterd., 1867.—Dippel, Mikroskop, Bd. II, p. 99 ff.—Sachs, Lehrb., p. 72.—Dippel, D. neuere Theorie über d. feinere Structur d. Zellhülle, etc. (Schr. d. Senckenbergischen Gesellsch., Bd. X, XI, 1875–78, p. 41 *ff.*).—Solla, Beitr. z. näheren Kenntn. der chem. und physikal. Beschaffenh. der Intercellulars. (Oesterr. bot. Zeitschr. 1879, pp. 341–353).—v. Höhnel, Notiz über d. Mittellamelle der Holzelemente, etc. (Bot. Zeitg, 1880, p. 450 *ff.*)—See also, in part, the writings cited on pages 319 and 333.

Under the conception of the middle lamella we are to understand the homogeneous, sharply-defined partition lying between two contiguous cells and which appears to be transformed from the primary cellulose membrane by chemical metamorphosis[48] and then often assumes certain conditions of solubility. It is always recognizable in wood tissue by being easily seen. With many anatomists the term "intercellular substance" is used as synonymous with "middle lamella." But Sachs and Wiesner distinguish between the two expressions and apply the former term to the jelly or pectin-like substance which sometimes lies between the cells and which is formed by a chemical metamorphosis in which more or less of the cell wall becomes homogeneous (endosperm of *Ceratonia siliqua*, the tissue of the Fuci, etc.).

The views of botanists concerning the production and chemical nature of the middle lamella or intercellular substance have greatly differed. Schacht[49] supposed the intercellular substance to be a binding cement between the cells distinct from the cellulose. According to Dippel[50] the middle lamella (which he calls the primary cellulose covering) is not homogeneous, but consists of two corresponding layers of cellulose and of one of intercellular substance lying between, which latter proceeds from the cambium walling of the tissue cells, which is formed rom a combination essentially different from cellulose but isomeric with it, and, indeed, of the daughter and not the mother cells of the tissue, which latter, as soon as they have fulfilled their function, are dissolved and reabsorbed. That combination essentially favors the dissolving of the contiguous coverings of the cells and allows, in consequence, transformations which are not peculiar to cellulose.

According to Wigand,[51] the intercellular substance arises from the intimate commingling of the primary cell walls, which view was also advanced by Sanio[52] who added that the intercel-

[48] A strict distinction must be made between the primary membrane and the middle lamella, which in some botanical hand books is not expressly done.

[49] Lehrb. d. Anat. u. Physiol. d. Gew., Bd. I, p. 129.

[50] Dippel, Mikroskop, Bd. II, p. 105 *f.*

[51] Wigand, Botan. Unters., p. 79.

[52] Sanio in Bot. Zeitg., 1860, p. 210 *ff.*

lular substance was altogether or partially lignified, and, indeed, this lignification takes place sometimes before that of the secondary cell layer. It colors yellow with chlor-iodide of zinc, but if the intercalated wood substance is removed, by boiling in potash the reaction of chlor-iodide of zinc will be that of cellulose. The views of the two last-named naturalists are current to-day.

The more recent investigations of Solla[53] teach that the intercellular substance or middle lamella, in the course of the development of the tissue, enters into different chemical as well as physical transformations. It is molecularly distinct from the adjoining layers of cell wall. The first foundation of the intercellular substance is either pure cellulose (cambium) or (at the point of the stem), a substance in which cellulose is afterwards traceable in the young permanent tissue. The intercellular substance of the young permanent tissue consists, as a rule, of cellulose. In perfectly formed permanent tissue cellulose is but rarely traceable (in many kinds of bast); commonly it enters into many metamorphoses and then exhibits toward the reagent a very different behavior. These metamorphoses lead finally sometimes to the complete separation of connected cells.

Reactions. Of the iodine reagents,[54] iodine and sulphuric acid, or chlor-iodide of zinc, produce in most cases a yellow coloring of the middle lamella; after previously boiling it in potash lye these reagents give a blue or violet tinted color (cellulose reaction).[55] This latter color, however, always appears at the outset when the middle lamella consists of cellulose. Partially lignified middle lamella shows a corresponding mixture of colors between yellow and violet. Boiling nitric acid in combination with ammonia often gives a strong yellow color to the middle lamella (Solla, v. Höhnel). Phloroglucin and indol behave toward the middle lamella very much as toward the lignified thickening layers of the cells. When the reaction takes place gradually the middle lamella colors before and more intensely than the adjoining layers. (See p. 337.)

[53] Solla in Oesterr. Bot. Zeitschr. 1879, pp. 341–353.
[54] Sanio, *l. c.*, Taf. VI, Figs. 10–12, 15.
[55] Sanio, *l. c.*, Taf. VI, Fig. 16.

Dissolving reagents, on the contrary, behave very differently toward the intercellular substance and its solubility is not proportionate to its age.[56] The rule is that cuprammonia, concentrated sulphuric acid and dilute chromic acid applied cold will not dissolve it (see p. 317), while concentrated chromic acid will do so with difficulty and Schultze's maceration mixture, easily.[57] The intercellular substance of very delicate tissue will sometimes be dissolved partially or altogether by the action of boiling water (for example in the parenchyma of the beet root, Wiesner). Acetic acid dissolves the intercellular substance of the potato after a long time,[58] tartaric and oxalic acid very slowly (Wiesner, Solla). Potash lye, nitric acid and muriatic acid dissolve it very rapidly (potato, pith of *Sambucus*). The middle lamella of wood is most rapidly dissolved in boiling nitro-muriatic acid and strong chromic acid. Potash lye slowly dissolves the intercellular substance of certain bast fibres as in the parenchyma of the beet root (Wiesner).

Intercellular substance may, in certain cases, undergo a pectose metamorphosis. Mulder[59] and Kabsch[60] first showed that pectose occurs in many cell walls; the latter also showed that in the boundary layer of the cells it is most intimately mingled with cellulose and appears as intercellular substance. Vogl[61] further found that in the root of the dandelion the intercellular substance is produced by the transformation of cellulose into pectose. Wiesner studied the appearance of the pectose bodies in the beet root and found, in agreement with Kabsch and Vogl, that the intercellular substance is the seat of the pectose which is principally a product of the transformation of the outer layer of the mother cell, but that not only parenchyma tissue, but also cambium, vascular and wood cells and likewise peridermal

[56] Wiesner in Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXII, 1 Abth. p. 201.

[57] According to Wiesner (Einleit. in d. techn. Mikrosp. p. 47) chromic acid will always dissolve the intercellular substance (see also Wiesner in Proceed. Imperial Acad. of Science, Vienna, Vol. LXII, part 1, p. 200). According to H. Müller, the intercellular substance of wood is dissolved by bromine water (official report of the Vienna World's Exposition, 1873, Brunswick, 1877, Vol. III, 1 part, 2nd half, p. 27 *ff*).

[58] Solla, *l. c.*, p. 344.

[59] Mulder, Physiol. Chem., p. 514.

[60] Kabsch in Pringsheim's Jahrb., Bd. III, p. 367.

[61] Vogl, Sitzungsber. d. K. Acad. Wiss. Wien, Bd. XLVIII, 2 Abth. 1863, p. 668 *ff*.

cells may contain pectose.[62] This pectose metamorphosis may lead in certain cases to the formation of jelly and to the loosening or separating of the individual cells. It may be mentioned also that pectic acid salts are detected in many plants; according to Fremy, calcium pectate is, in many of the tissues, the cementing medium of the cells. According to Maudet it is an element of the pith of *Aralia papyrifera*. According to Gireaud pectic acid is found in large quantities in gum tragacanth.[63]

The presence of pectose substances is demonstrated by the cell walls swelling in boiling water and potash lye, and dissolving in the latter. According to Poulsen[64] cuprammonia will precipitate in tissue containing pectose a copper pectinate, which in thin sections will still remain after the entire disintegration of the rest part of the membrane.

The methods of investigating intercellular substances with reagents have been sufficiently given in treating of lignin. Should we wish to dissolve these substances by heating the reagent, the operation may be conducted with a watch-glass which should not be heated over a free flame, but, following the process of Sanio,[65] should be placed upon a thin plate of iron and this heated till the contents of the glass boil.

5. Corky Cellulose, Suberin.

(Including Cutin, Pollenin.)

Literature. Kroker, De plantar. epidemide observ. Vratisl., 1833.—Mohl, Unters. über d. Entwickl., des Korkes u. d. Borke auf der Rinde der baumart. Dikotyl. (Verm. Schr., pp. 212–232; auch Diss. aus d. Jahre, 1836).—Mohl, Unters. über d. Lenticellen (*id.*, pp. 233–245; auch Diss. vom. Jahre, 1836).—Mohl, Ueber d. Cuticula der Gewächse (*id.*, pp. 260–368; auch Linnæa, 1842).—Fritsche, Ueber den Pollen, Petersbg., 1837.—Nägeli, Entwicklungsgesch., d. Pollens, etc., Zurich, 1842.—Cohn, De Cuticula. Vratisl., 1850.—Schacht,

[62] Wiesner, ibid, Bd. L, 2 Abth, 1864, p. 450.
[63] Husemann, Pflanzenstoffe, Bd. I, 1882, p. 186.
[64] Poulsen, Botan. Mikrochem., p. 57, Trans. pp. 15, 91.
[65] Sanio in Bot. Zeit., 1860, p. 211, Anm.

D. Pflanzenzelle, p. 239.—Hanstein, Ueber d. Bau u. d. Entwickl. d. Baumrinde, Berlin, 1853.—Fremy, Recherches chim. sur la cuticule (Comptes rendus de Paris, t. XLVIII, 1859, p. 667, *ff.*).—Sanio, Ueber d. Bau. u. die Entwickl. des Korkes (Pringsheim's Jahrb., Bd. II, 1860, pp. 39–108).—Schacht, Ueber d. Bau einiger Pollenkörner (*id.*, pp. 109–159).—Pollender, die Chroms., ein Lösungsmittel für Pollenin u. Cutin (Bot. Zeitg., 1862, p. 405).—Faivre, Sur les plaies d'écorce par incis. annul. et sur leurs effets, etc., Paris, 1864.—Flückiger, Lehrb. d. Pharmakogn. d. Pflanzenreiches, Berlin, 1867, p. 336.—De Bary, Ueber d. Wachsüberzüge der Epidermis (Bot. Zeitg., 1871, p. 128, *ff.*)—Pfitzer, Beitr. z. Kenntn. d. Hautgewebe d. Pfl. (Pringsheim's Jahrb., Bd. VII, p. 532, *ff.*, Bd. VIII, p. 73, *ff.*)—Hegelmaier, Ueber d. Bau u. die Entwickl. einiger Cuticulargebilde (*id.*, Bd. IX, p. 286, *ff.*).—Haberlandt, Ueber d. Nachweisung der Cellulose im Korkgewebe (Oesterr. bot. Zeitschr., 1874, pp. 229–234).—Müller, R., Die Rinde unserer Laubhölzer. Bresl., 1875.—Tschistiakoff, Ueber d. Entwicklungsgesch. des Pollens v. Epilobium angustifolium (Prings. Jahrb., Bd. X, p. 7–45).—v. Höhnel, Ueber den Kork und verkorkte Gewebe überhaupt (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXVI, 1 Abth., 1877, p. 507–562; *cf.* auch Bot. Zeitg., 1877, p. 783, *ff.*).—v. Höhnel, Ueber d. Cuticula (Oesterr. Bot. Zeitg., 1878, No. 3, u. 4).—Niggl, D. Indol, ein Reagenz auf verholzte Zellmembranen, p. 9, *f.*

Corky or cuticularized cellulose is distributed through the well-known cork layer, which is often solid, free from intercellular spaces, mostly consisting of not very much thickened cell layers and their derivatives, also in the endodermis, and in that fine continuous coating which is drawn over the outer walls of the epidermis cells, and finally in the outer inclosing sheath of pollen grains and many spores. According to De Bary's[66] and especially v. Höhnel's[67] investigations the suberization does not seize upon all portions of the cork cell region, but it is

[66] De Bary in Bot. Zeitg., 1871, p. 128, *ff.*

[67] v. Höhnel in Sitzungsber. d. K. Acad. Wiss. Wien, Bd. LXXVI, 1 Abth., p. 507.

limited to certain definite, often sharply marked, zones. According to v. Höhnel, almost every cork cell wall (exclusive of many young cork cells of the *Coniferæ*), which belongs to two neighboring cells, consists of the following five lamella: (1) of a middle strongly lignified plate, which is not distinguishable from the middle lamella, or is only partially lignified; (2) of two suberized layers which lie on the two sides of this one; (3) of two cellulose layers which lie next to the two last and also to the cell space, and which are more or less strongly lignified.

The cork substance forming the suberin layer is as little known in respect to its chemical nature as is lignin. According to Mitscherlich, Döpping and others, cork substance is distinguished by containing 1.50 per cent to 2.3 per cent of nitrogen, while according to v. Höhnel there are no grounds for supposing it to contain nitrogen, since albuminous substances have never anywhere been detected in suberin. But suberin contains from 73 to 74 per cent of carbon and 10 per cent of hydrogen (by which it follows that it must contain from 16 to 17 per cent of oxygen). It is insoluble in boiling alcohol and stands in its chemical as in its physical nature between wax and cellulose. De Bary[68] has shown that frequently, perhaps always, in the formation of cuticle, a molecular intercalation of wax takes place. A very characteristic physical quality of the cork lamella is that it is almost entirely impermeable by diosmosis, as Sanio,[69] by a series of very striking experiments, has already proved.

As in other modifications of cellulose, so in the suberine lamella, pure cellulose may often be detected when the "incrusting substance" has been removed by a process already described on pages 318 and 331. The first who directed attention to this characteristic were Mohl[70] and Hofmeister[71]. The former noticed the cellulose in the cork of a flask after

[68] De Bary in Bot. Zeitg., 1871, p. 593, *ff.*

[69] Sanio in Pringsheim's Jahrb., Bd. II, p. 54, *f.*—See also De Bary, *l. c.*; Hanstein in Bot. Zeitg., 1868, pp. 708, 748; Behrens in Flora, 1879, p. 374, *f.*

[70] H. v. Mohl in Bot. Zeitg., 1847, p. 497.

[71] Hofmeister in Ber. d. K. Sächs Gesellsch.d . Wiss. Leipzig, Bd. X (1858), p. 21.

maceration in potash lye. The latter showed that the laminated cuticular layer of epidermis cells of *Hoja carnosa* gave a very distinct blue coloring with iodine reagent when it had been excluded from the air and treated for two or three weeks with concentrated potash lye. The same reaction appears in the cuticle of the leaves of *Orchio morio* after previous treatment with concentrated sulphuric acid. "The principal reasons for including cuticle among those membranes which are essentially different from cellulose falls to the ground with this proof."[72] According to De Bary[73] the cuticular substance of the leaves of *Klopstockia* is very easily destroyed by a warm ten per cent solution of potash whereof the pure cellulose walls remained behind. Haberlandt, who has made the most searching investigation of the occurrence of cellulose in cork found that the test may be made by maceration in chromic acid, and Schultze's mixture, but preferably by boiling the section to be tested in potassium chlorate and nitric acid, and then before the section quite falls apart treat it for some moments with boiling potash lye. Then after washing it out with water the membranes of the separated tissue will be colored an intense blue by chloriodide of zinc and be dissolved by cuprammonia.[74]

The suberized parts of the membrane may be easily recognized as such by some characteristic reactions. Like the middle lamella it is perfectly insoluble in cuprammonia and concentrated sulphuric acid. The latter often colors the cuticularized extine of many pollen grains usually a beautiful rose-red,[75] seldom yellow. Acetic acid causes the extine of many spores of ferns to swell.[76]

Concentrated chromic acid itself will not dissolve suberin, or if at all with the greatest difficulty; v. Höhnel uses this therefore as a test of suberin (chromic acid reaction):[77] use a pure quite concentrated solution. It causes the suberized membrane to stand out clear and distinct while the rest part of the tissue first

[72] See also v. Mohl, Vermischte Schr., p. 263.
[73] De Bary, *l. c.* p. 578.
[74] Haberlandt, in Oesterr. Bot. Zeitsch., 1874, p. 232, *f.*
[75] Schacht in Pringsheim's Jahrb., Bd. II, p. 134, etc. Taf. XVIII, Figs. 10, 11, 14, 15, 31, 32.
[76] Fischer v. Waldheim in Pringsheim's Jahrb., Bd. IV, p. 375.
[77] v. Höhnel, *l. c.*, p. 526.

becomes gradually more indistinct and then entirely disappears. Suberized membrane is dissolved by chromic acid, as previously noted only with much difficulty but after treatment by it for eight or ten hours it becomes transparent. Very strongly suberized membrane holds out, however, for a week, but finally becomes transparent. Wash out the acid and again it becomes dark and distinct as before. Cuticularized membranes have the same characteristics.

Iodine with sulphuric acid as well as chlor-iodide of zinc colors suberized cellulose yellow or brown or deep brown itself. According to Mohl[78] if the cuticle is impregnated with iodine it is colored a deep yellow or brown. If the specimen is treated with sulphuric acid the cuticle is dissolved off and can be seen very well. Sometimes by this process it becomes a still darker brown.[79] The extine of the pollen grain colors with iodine and sulphuric acid quite the same way.[80] So also do many spores, as for example, fern spores.

Indol with sulphuric acid (see p. 339) leaves suberized cells, as in the cuticle, quite uncolored,[81] at least the suberized lamellæ never show any coloring, while in the walls of old cork cells a red coloring is noticeable after this treatment. But, as can be shown in very thin sections, only the middle lamella is stained. Young cork cells show no reddening. Concentrated potash lye produces no noticeable change in cork tissue except a very faint yellow coloring. But by holding the slide over a small flame, slowly heating it but not quite to the boiling point, the color becomes darker, the membrane itself a little swollen and at least a definite layer of the wall assumes a granular appearance (pure cellulose membrane only swells but for the rest maintains a smooth surface). By boiling the granulization becomes more pronounced and in most cases the granular and

[78] v. Mohl, Verm. Schr., p. 261.— For particulars concerning the coloring of cuticle see pp. 260–267, and compare with illustrations in Tables IX and X.

[79] According to Hofmeister (Ber. Sächs, Gesselsch., Leip. Bd. X, p. 21), the cuticle of the seed of *Linum usitatissimum* behaves in a characteristic manner. By treatment with iodine and dilute sulphuric acid it is colored blue the blue bordering on the black. Adding concentrated sulphuric acid changes the color to yellow. Washing out the acid with water restores the blue color.

[80] Schacht, *l. c.*, p. 103–168.

[81] Niggl, *l. c.*, p. 9.

variegated substances protrude from the membrane. If now the section be washed with water the granular masses will be for the most part destroyed. It now becomes evident that every cell wall of cork, however thin it may be, consists of three membranous lamellæ (see p. 348), a middle one in common and two which belong to the adjoining cells, which lamellæ are often separated from each other by a wide space between. These spaces were originally filled with this granular mass. Suberized cells treated with a cold concentrated solution of potash take on a yellow color while all other cell membranes remain almost or altogether uncolored. By heating, the yellow color of the first becomes more intense, and that of the latter when there is any, more pale (potassium reaction, v. Höhnel).[82]

By boiling the section under investigation in Schultze's mixture (see p. 163) the suberized membrane becomes very indistinct, while the rest, even strongly lignified tissue itself, gradually becomes transparent. Cuticle and cuticularized tissue behave in the same way. Warmed under a cover-glass a violent development of gas takes place and then the suberized tissue alone remains. Now wash out the Schultze's mixture and add alcohol and then ether and the whole becomes hyaline. But if the heating is carried still further the membranes suddenly swell and melt together into masses which finally become quite globular, consist of ceric acid, and are soluble in hot alcohol, ether, benzole, chloroform and dilute potash lye. To recognize slightly suberized tissue, lay the section a short time in cold Schultze's mixture wash out and add potash lye. By the former the suberized membrane becomes somewhat more distinct and by the latter it takes an ocher yellow color and becomes crumbly. If this does not immediately happen slightly warming commonly helps it on. At the same time the potash lye produces a further clarification of the suberized membrane (ceric acid reaction, v. Höhnel).[83]

[82] v. Höhnel, pp. 522–524.
[83] v. Höhnel, *l. c.*, pp. 524–526.

6. Fungus Cellulose.

Literature. Schacht, Die Pflanzenzelle, p. 13.—Dippel, D. Mikroskop, Bd. II, p. 7, *f.*—De Bary, Morphology der Pilze, Flechten und Myxomycetin (Hofmeister, de Bary, Sachs, Handbuch d. Physiol. Botan., Bd. II, p. 7, *ff*).—Poulsen, Bot. Mikrochemi, p. 51 (Trans. p. 79).—Richter, Beiträge z. Genaueren Kenntn. der chem. Beschaffenheit d. Zellmembranen bei den Pilzen (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXXIII, 1 Abth., 1881, pp. 494–510). [Ber. Deutsche Bot. Gesellsch. I (1883), pp. 288–308 (1 pl.), Jour. Roy. Mic. Soc., Vol. III, part V, p. 676.]

The membranous substance which forms the walls of the hyphæ of fungi and lichens, fungus cellulose, was, until the recent investigations of Schacht, Dippel and De Bary, looked upon as an entirely different substance to cellulose, since it had not been possible before that by any known medium of maceration to remove the incrusting substance so as to produce by iodine reagents the cellulose reaction. According to Schacht and De Bary the fungus walls themselves were not colored blue with iodine and sulphuric acid after boiling in potash lye, nor indeed after treatment with Schultze's mixture or chromic acid. For the rest it was already known by De Bary and others that many plant membranes become blue by iodine or chlor-iodide of zinc without any other previous treatment. In *Mucor* it was demonstrated that in the young state the cell walls were colored blue with iodine and sulphuric acid, but in the older stages they remained colorless.

In later investigations Richter opposed the view that fungus cellulose is essentially different from true cellulose. He succeeded in removing the incrusting substance from the fungus *Polyporus*, after long and numerous treatments of the fungus tissue with water, hot potash lye, acetic acid, alcohol, ether and again water, and then in producing the cellulose reaction with chlor-iodide of zinc. The same results were produced with fungi and lichens by macerating the tissue continuously

for two or three weeks in potash lye frequently changing the macerating liquid.[84] The fungus membrane thus purified was colored a rose red to a violet by chlor-iodide of zinc; it also appeared to be dissolved by cuprammonia but this could not be definitely verified. Richter[85] did not succeed in demonstrating any lignification of these membranes by means of aniline sulphate or phloroglucin, not even in the lichen itself.[86] On the other hand, he demonstrated the most distinct suberization in *Daedalea quercina* by means of the ceric acid reaction (see p. 351).

The purification of the membranes of fungus cells so as to get the cellulose reaction is generally accomplished only with the greatest difficulty, and the utmost patience is necessary. Richter[87] found by his studies that the fungus cellulose is but common cellulose with an admixture of foreign matter, perhaps albuminous substances; that a fungus cellulose in the sense of De Bary does not exist.

In its natural state fungus cellulose is distinguished by its extraordinary resistance to the different reagents. It is perfectly insoluble in cuprammonia, can be scarcely touched by cold potash lye, muriatic acid and Schultze's mixture. Concentrated sulphuric acid destroys it only with the greatest difficulty. On the contrary, it should be distinctly stated that many fungus membranes are soluble in muriatic acid.

[Pringsheim has further investigated the peculiar granules long since observed by him in the fertilizing tubes and oögonia of the Saprolegnieæ and described by Zopf as amœbæ. They are found in the fertilizing tubes at all ages. While young they are flat disk-shaped or polyhedral plates with rounded corners composed of a dense homogeneous substance. They vary greatly in size and form. They gradually become stratified and at last as regularly and completely as starch grains. They are abundant also in the oögonia and a few grains occur in other parts of the plant.]

[84] Of lichens those must be selected which have the least possible amount of lichenin in them since this gives the same reaction as cellulose (Richter, *l. c.*, p. 503. See above p. 270).

[85] Richter, *l. c.*, p. 505, *f.*

[86] On the contrary, see Burgerstein above p. 283, and Niggl above, p. 287.

[87] Richter, *l. c.*, p. 510.

[The structure, mode of development and chemical properties of these substances show that they are neither organs of reproduction nor independent parasitic organisms but are a special modification of the cell contents. The stratification indicates a close resemblance to other bodies of this character. They are, however, not colored blue by iodine nor do they take any other color but that of the iodine itself. They are completely insoluble in all ordinary solvents of oils and resins, even in absolute alcohol and ether. Nitric acid either with or without ammonia or potash produces no effect on them nor does Millon's reagent. They have no power of taking up coloring substances except under special circumstances. Caustic alkalies cold produce no visible effect on these bodies and very little change is effected by dilute or concentrated nitric or hydrochloric acid at common temperature. In moderately concentrated sulphuric acid they dissolve rapidly and completely, at the ordinary temperature, as also in solutions of zinc chloride when not too dilute. They do not dissolve in cuprammonia even after long treatment.]

[These reactions show that the bodies in question belong neither to the proteinaceous cell contents, nor to the series of oils and resins, but that they are composed of a substance closely allied to cellulose which has been separated from the protoplasm in a granular form. It is perhaps identical with so-called "fungus cellulose" and with the "fibrose" of Fremy, and Pringsheim proposes for it the term "cellulin." Its special chemical characteristic is its remarkable solubility in dilute sulphuric acid, and in an aqueous solution of zinc chloride.]

The stratification of the cellulin grains is concentric around a nucleus of denser substance. They grow, however, to a considerable size before any stratification is evident. Compound grains are not uncommon. A common mode of multiplication is by a kind of budding not dissimilar to that of torula.

[When the oöspores are formed out of the protoplasmic contents of the organism, an unused residue remains behind, which is the substance out of which the cellulin grains are subsequently developed. This substance is morphologically identical with

A TABLE OF THE MOST IMPORTANT CELLULOSE REACTIONS.

		Iodine water.	Chlor-iodide of zinc.	Iodine and sulphuric acid.	Cuprammonia.	Potassium hydroxide.	Concentrated sulphuric acid.	Nitric acid.	Chromic acid.	Schultze's mixture.	Phenol-muriatic acid.	Aniline sulphate.	Phloroglucin.	Indol.	
1	Cellulose. Cell substance.	Yellow to brownish.	Violet.	Blue.	Dissolves.	Swells up	Dissolves.	Swells up.	Dissolves.	Dissolves.	Uncolored.	Uncolored.	Uncolored.	Uncolored.	1
2	Lignin. Wood substance.	Yellow.	Yellow.	Yellow. to brownish.	Insoluble.	Dissolves.	Dissolves.	Soluble.	Dissolves.	Is easily dissolved.	Green.	Yellow or gold yellow.	Purple red.	Cherry red.	2
3	Middlelamella. Intercellular substance.	Yellow.	Yellow.	Yellow.	Insoluble.	Insoluble or soluble.	Insoluble or soluble.	Mostly ins. yel'w by bl'g n. a. & the addit'n of ammonia afterwards.	Insoluble or soluble.	Is easily dissolved.	Green.	Gold yellow.	Purple red.	Cherry red.	3
4	Suberin. Cork substance.	Yellow or brownish.	Yellow or brown.	Brown.	Insoluble.	Cold, insoluble; by boiling, it comes out in drops.	Insoluble.	Transformed into a yellow homogeneous mass.	Insoluble or very slightly soluble.	Gives ceric acid reaction.	Uncolored.	Uncolored.	Uncolored.	Uncolored.	4

the "periplasm" of the *Peronosporeæ* out of which the exospores of the oöspore is formed. *Quoted from Journal Royal Microscopical Society, l. c.* A. B. H.]

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Having finished our presentation of the microscopical examination of cellulose we present the various reactions in a tabular form for greater convenience in using them. It will be very easy to identify by means of this table the different kinds of cellulose which are subjected to examination. Muculent, as well as fungus cellulose has been excluded from this schedule since by their peculiar outward appearance, or the manner of their occurrence, they are immediately recognized as such. In the species of cellulose cited we have had in mind only the pure types, and in those cases where it has been transformed into another or mingled in the membranous layer with another (as, for example, in the partial lignification of suberin lamellæ) the reader will have to consult the foregoing chapter.

II. STARCH, AMYLUM.

Literature. Fritsche, Ueber das Amylum (Poggendorf's Ann. Bd. XXXII, 1834, p. 129, *ff.*)—Payen, Compos. Elém. de l'Amidon de diverses Plantes, etc. (Annales de Chim. et de Phys., t. LXV, 1837, p. 225 *ff.*)—Nägeli, Bläschenfg. Gebilde im Inhalte d. Pflzelle, 7, Stärkebläschen, Stärkekörner (Zeitsch. f. wiss. Bot. v. Schleiden und Nägeli, Heft 3, 4, p. 117, *ff.*)—Mohl, D. veget. Zelle (Wagner's Handwörterb. Bd. IV, 1851, p. 207.)—Maschke in Erdmann's Archiv, f. prakt. Chem., 1852, 2, p. 400.—Walpers, Beiträg. z. Kenntn. d. Amylums (Flora, 1852, p. 689 *ff*, 705 *ff.*)—Hartig, Ueber die Bau des Stärkemehls (Bot. Zeitg., 1855, No. 52 — Nachtrag dazu ebendaselbst, 1856, p. 349 *ff.*)—Melsens, L'Institut, 1857, p. 161.—Cramer, Verh. d. Kupferoxydammoniaks, z. Zellmembran, Stärke Inulin, etc., Zurich, 1857.—Nägeli, D. Stärkekörner, Zurich, 1858. —Nägeli u. Cramer, Pflanzenphysiol. Unters. II (1858), p. 113

ff. 181 *ff.*—Hartig, Entwicklungsgeschich. d. Pflkeims, 1858, pp. 88, 155.—Mohl, Unters. d. Pflanzengewebes mit Hilfe des polarisirten Lichtes (Bot. Zeitg., 1858, p. 1, *ff.*)—Sachs, Ueber einige neue Reactionsmethodin (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. XXXVI, 1859, p. 5, *ff.*)—Mohl, Ueber d. vorgeblichen Gehalt d. Stärkekörner an Cellulose (Bot. Zeitg. 1859, p. 225 *ff.* 233 *ff.*)—Sachs, Ueber d. Auftreten d. Stärke bei d. Keimung ölhaltiger Samen (*id.* p. 177 *ff.*, 185 *ff.*)—Sachs, Mikrochem. Unters. (Flora, 1862, p. 299 *ff.*)—Nägeli, Ueber d. Reactionen v. Jod auf Stärkekörner u. Zellmembranen (Ber. d. Bayer. Acad., 1862, 1863, Bd. I, p. 161 *ff.* 483 *ff.*)—Nägeli, Ueber d. Chem. Verschiedenh. d. Stärkekörner (*id.* 1863, Bd. II, p. 272, *ff.*)—Sachs, Ueber d. Entstehung der Stärke in den Blättern (Monatshefte d. Annalen d. preuss. Landwirthsch., 1863)—Sachs, Ueber d. Stoffe welche das Material z. Wachsthum der Zellhäute liefern (Pringsheim's Jahrb., Bd. III 1863, p. 183, *ff.*)—Mulder, Physiol. Chem., p. 217.—Sachs, Handb. d. Experimentalphysiol. d. Pflanz. p. 412, *ff.*—Hofmeister, Handb. d. physiol. Bot., Bd. I, p. 387, *f.*—Dippel, D. Mikroskop, Bd. II, p. 24.—Nägeli u. Schwendener, D. Mikroskop, p. 512, *ff.*—Sachs, Lehrb. d. Bot., p. 59 *ff.*—W. Nägeli, Bietr. z. näheren Kenntniss der Stärkegruppe, 1874.

Starch or Amylum ($C_6H_{10}O_5$ or, according to W. Nägeli, $C_{36}H_{62}O_{31}$) is isomeric with cellulose and is almost universally distributed as solid contents of plant cells. It occurs in all the phanerogams but has not yet been observed in the fungi and some families of algæ. It is the first visible product of assimilation by the chlorophyll grains, and is formed within them.* At certain times in the life of the plant it is changed into the form of a fluid isomeric carbo-hydrate (glycose) and circulates

*J. Boehm (in Botan. Zeitg. XLI (1883) pp. 33-38, 49-54) contests the ordinary view that starch formed in the chlorophyll grains is a direct result of the decomposition of carbon dioxide. He believes it to be in many cases formed from sugar or other organic substances which have found their way into the chlorophyll grains. Leaves and pieces of the stem of the scarlet runner containing no starch were exposed to the action of a solution of sugar when they were found, after 24 hours, to contain an abundance of starch, the quantity depending on the concentration of the sugar solution. This author thinks therefore that sugar and not starch is the first demonstrable product of the decomposition of carbon dioxide. Quoted from Jour. Roy. Mic. Soc., Vol. III, No. III, pp. 388-90. A. B. H.

to other parts of the plant to be again transformed into starch in the parenchyma, the medullary rays of the stem, rhizomes, tubers, bulbs and seeds. There, after having been inactive for a long time, as a reserve substance, it will furnish rich material for the building up of new organs in a new period of vegetable growth after first being changed into sugar and dextrine. The vessels, as it appears, are always free from starch.

FIG. 132.

It is very often easy to recognize starch as such by its outward appearance. It forms roundish, transparent, colorless, elliptical, egg-shaped, irregular grains, simple or compound, of from 0.001 mm. to 0.15 mm. in diameter. The starch grain is laminated, the laminæ lying about a mostly excentrically placed nucleus which, in the young grain, is filled with aqueous substances and in the old grain with air. The layers are optically distinct

by reason of their differing contents of water (Fig. 121, p. 263). When the lamination is not easily seen the addition of dilute chromic acid will make it more distinct.[88]

Alcohol, on the contrary, makes the lamination less distinct. Starch grains disperse the light into two rays of unlike velocity which polarize at right angles to each other. Therefore they appear bright in the polarizing apparatus (see p. 133) with the prisms crossed, bright with a dark cross running over them and passing through the before mentioned nucleus (Fig. 132). Starch grains behave like double refracting crystals and we assume, therefore, that they consist of a mixture of anisotropic substances.

Of all reagents employed in testing starch, iodine solutions hold the first place. Colin and Gaultier de Claubry observed as early as 1814, that a solution of iodine gave a blue coloring to starch. By it the starch was changed to an iodine amylum. The color which iodine imparts to starch seems to depend upon the kind and concentration of the solution used, and will be violet, indigo-blue, or a deep dark blue. The color is commonly a full indigo blue; a pure blue does not occur (Nägeli). All iodine reagents produce this coloring by the intercalation of iodine atoms in the starch grain. The reaction is so distinct that starch granules of but 2 to 3 μ in diameter are certainly recognizable as such. The intensity of the bluing is not proportional to the iodine contents of the solution. The quantity of the intercalated iodine conditions the intensity of the color of the starch grains but it can produce no distinction in the tone of the color (Nägeli).

The bluing takes place only when the starch grains contain water (Mohl). An alcoholic solution of iodine colors anhydrated amylum very slowly a brown yellow. If the iodine alcohol is mixed with much water it gives a brown or violet tone (Nägeli).

By the use of potassium iodide of iodine the bluing takes place very quickly, more slowly with iodine water and most slowly

[88] Dippel, D. Mikroskop, Bd. I, p. 276.

of all with iodine glycerine. The last named reagent should be used in studying the very gradual effect of iodine.[89] According to the investigations of Nägeli,[90] potato starch in water colors on the addition of some iodine at first a bright blue, but afterward an intense indigo blue. If the iodine is removed, the color gradually disappears; but, before it becomes quite colorless, it shows again the bright violet. If the iodine reagent contains no other substance than iodine (potassium iodide, hydriodic acid, etc.), the starch will retain its blue color on drying. But in other cases, if it has taken in another substance it will change color through violet, red and orange to yellow, in exact relation to the quantity of the absorbed substance. Starch grains in zinc chloride, by the addition of a splinter of iodine, take the color with extraordinary slowness. Before the grain swells it becomes red violet or pale violet, and afterwards a pure blue. Concentrated solution of zinc chloride like sulphuric acid disintegrates the starch grains into many small particles. If the solution of chlor-iodide of zinc is made very dilute with water, the starch grains will not swell up, and will become blue violet or indigo blue.

"In exactly the same treatment with iodine reagents the different parts of a starch grain and the different kinds of starch behave differently; perhaps the one having a somewhat stronger affinity for the iodine colors more rapidly, and so takes on a somewhat different tone of color.

The particular grain of starch, or the particular layer of a starch grain, gives with iodine a different color according to the nature and the quantity of the foreign substances which have permeated it (water, acids, salts, indifferent organic combination, etc.), according to whether these substances enter the starch grains before or after the iodine, and according to whether the iodine still keeps its original arrangement, or is already making preparations to leave the starch.

The colors which the iodine can produce in the starch are indigo, violet, red, orange and yellow. They depend on a pecu-

[89] Hartig, *l. c.*; see for the rest, Nägeli, Bayer. Acad. 1863, I, p. 188.
[90] Nägeli, *l. c.*, p. 161 *ff.*

liar arrangement of the particles of iodine and are in general nothing else than what is recognized in the iodine itself in a solid, liquid and gaseous state.

Of the different starch and iodine combinations the blue corresponds to the strongest affinity, the yellow to the weakest. When the iodine enters the starch it always assumes that molecular arrangement which the greatest possible affinity requires, under the given conditions; if, on the contrary, it is compelled to leave the starch by some other force it previously changes its molecular constitution in the manner that corresponds to the weaker affinity. The presence of water always conditions the color corresponding to the stronger absorption and intercalation of the particles of iodine, the presence of some other substance on the contrary produces that color which corresponds to the weaker affinity."[91]

Concerning the behavior of some other substances towards starch the following may be mentioned.

Bromine, with water, colors starch grains a pure orange yellow.

Starch is insoluble in water, alcohol and ether, insoluble but swelling in concentrated iodine solutions, chlor-iodide of zinc, bromine solutions, calcium chlorate and cuprammonia (the addition of water restores the grains to their original volume), soluble in all, even the very dilute, mineral acids. The latter finally change it into combinations of the dextrine group. Cuprammonia colors starch grains dark blue. If starch in water, potash lye, dilute mineral acids and certain organic acids be warmed to about 50° it becomes structureless and then the well-known paste-forming process begins.

According to the investigations of Nägeli the starch grain consists of two essentially different components, granulose which gives the characteristic iodine reaction, and starch cellulose.

If starch be digested by a gentle heat with saliva fluid, or with pepsin (Melsens), or treated with chromic acid, the granulose, that component of the starch grain which is blued with iodine will be withdrawn, and the element which remains, the

[91] Nägeli, *l. c.*, pp. 176, 197, 198.

delicate framework of starch cellulose, will not then color blue with iodine reagents, but for the most part the same as cellulose (Nägeli).[92] Iodine water, or fresh iodine alcohol stains it if at all, a weak, pale copper red. After drying, potassium iodide of iodine, chlor-iodide of zinc and iodine alcohol produce a violet to an indigo blue color (Nägeli). Micro-chemistry distinguishes starch cellulose from many other kinds of characteristic cellulose by its easy solubility in potassium hydroxide and chlor-iodide of zinc solution. Starch cellulose dissolves in cupram-monia (v. Mohl).

It still remains to be shown how starch is tested when it is enveloped in protoplasmic substances, or when it is found within the chlorophyll grains.

A. Starch mingled with Proteid Plasmic Substances.[93]

In very young and small-celled parenchyma tissue which is developed from the primary meristem of the vegetation point, starch is almost never to be discovered by means of iodine alcohol or potassium iodide of iodine. If chlorophyll is not present a thin section is warmed in the strongest potassium hydroxide, or left to lie in it cold for a considerable time, then washed with water and neutralized with acetic acid and iodine alcohol much diluted with water added. Then with strong magnification either swollen blue grains will be seen in the yellow plasma or a blue paste. In the latter case it will naturally be impossible to determine if the starch occurs here in grains or in solution perhaps intimately united with the nitrogenous contents of the plasma.

If the plant tissue which has been washed out contains much

[92] See on the other hand what H. v. Mohl says in objection to this (Ueber den angeblichen Gehalt der Stärkekörner an Cellulose; Bot. Zeitg. 1859, 225, *ff*).

[93] Sachs, Concerning the substances which supply the material for the growth of the cell wall (Pringsheim's Jahrb. III, 1863, p. 183, *ff*.)— Behrens, Die Nectarien der Blüten (Flora, 1879, p. 244).

protoplasmic proteid substance and in this small imbedded grains of starch (transitorily) as in the organs of secretion, or, in the guard-cells of the stomata, the blue reaction of the starch by the use of the iodine reagent will be concealed by the yellow brown of the nitrogenous substances. In this case in order to make the starch visible lay the preparation in dilute potassium hydroxide which will dissolve the greater part of the proteid substance. Wash with distilled water, neutralize as much as is necessary with weak acid and then add the potassium iodide of iodine solution. With many preparations this procedure is not necessary since sometimes the starch colors before the plasma. In such cases a dilute iodine solution may be suitably employed.

B. Starch in Chlorophyll Grains.[94]

The green color of the chlorophyll conceals the blue reaction of the iodine upon the starch grains contained in the chlorophyll, and the chlorophyll grains receive a verdigris green stain from the iodine. Very small granules of starch thus embedded appear of a brown color after treatment with iodine reagents (v. Mohl). In such cases in order to make the starch grains visible it is necessary to lay the preparation in absolute alcohol and set it in the direct sunlight to extract the green chlorophyll coloring matter. Whenever the section is ready treat with potassium hydroxide to swell the starch grains, wash out the alkali with water and add solution of iodine (Böhm). Or treat the bleached section with boiling potassium hydroxide, wash out, neutralize with weak acid and add dilute iodine solution. Then we get the swollen starch grains colored a distinct violet blue. Sometimes the preparation becomes still more beautiful when it has lain for a day or two in glycerine. In filamentous algæ (Conferva) it is enough often to add the iodine alone to make the large starch grains visible through the thin layer of chlorophyll (v. Mohl).

[94] H. v. Mohl, Vermischte Schriften, p. 355.—H. v. Mohl in Bot.Zeit., 1855, pp. 110, 111.—Böhm in Sitzungsber. d. K. K. Acad. Wien, 1857, p. 21.—Sachs in Flora, 1862, p. 166.—Sachs in Pringsheim's Jahrb. Bd. III, 1863, pp. 186, 200.

III. DEXTRINE.

Literature. Sachs, Ueber einige neue mikrosk.-chem. Reactionsmethoden (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. XXXVI, 1859, p. 8, *ff.*)—Sachs, Mikrochem. Untersuchungen (Flora, 1862, p. 289, *ff.*)—Sachs, Ueber d. Stoffe, welche das Material zum Aufbau der Zellhäute liefern (Pringsheim's Jahrb., Bd. III, 1863, p. 183, *ff.*)—Dippel, Mikrosk., Bd. II, p. 19.—Nägeli u. Schwendener Mikrosk., p. 475, p. 525.—W. Nägeli, Die Stärkegruppe.—Poulsen, Bot. Mikrokemi, p. 54 (Trans. p. 87).

Dextrine $C_6H_{10}O_5$ or $C_{12}H_{20}O_{10}$ is, in the dry state, an amorphous gum-like mass, in solution—as it occurs in the cells—a colorless clear fluid which is produced by the transformation of starch. This transformation seems to proceed in this way: The granular starch appears first to be changed into a starch solution (amylodextrine), and from this is produced the characteristic dextrine. It has not yet been possible to trace microscopically this transformation. A wood dextrine produced from cellulose should also be mentioned (Béchamp).

The single method known at the present time for microscopically testing dextrine is that introduced by Sachs, by means of Trommer's reagent (copper sulphate and potash solution, see pp. 293 and 325). This reagent is useful for testing at the same time the other soluble carbohydrates of the cell contents—grape sugar, cane sugar, etc.—it often likewise affords the means of studying the relative quantity of these elements as well as their distribution in the interior of plants.

Method (Sachs).[95] In order to prove that a given part of a plant contains dextrine (or sugar or both) we should make both a transverse and a longitudinal section which is at least two or three cell-layers thick and lay them in a vessel containing a concentrated copper sulphate solution. While they are here absorbing the salt, a small porcelain dish holding about 5 or 6 cc.

[95] Sachs in Pringsheim's Jahrb., Bd. III, p. 187, *ff.*

filled with strong potash lye should be heated to boiling over the flame; then with the forceps take the section out of the copper sulphate solution and dip it several times in a vessel of water and lay it immediately in the hot alkali.

If the cells contain grape sugar or dextrine there will be produced almost instantly or after a few seconds sometimes, a beautiful opaque red coloring which approaches now to brick red and now to yellow. This coloring comes from the precipitate cuprous oxide produced in the cells which under high magnification appears in the form of small, roundish grains in the cells. In most cases the reaction is so brilliant that the unaided eye can distinguish the presence of the precipitate, even if it occurs only in single cells. Still it is always advisable to accept the aid of the magnification. If, on the contrary, the section contains cane sugar, a beautiful, sky-blue color is produced on dipping the section in potash after it has been previously saturated with the copper salt solution and washed, which color belongs to a clear fluid contained in the cells. There is produced no precipitate of red cuprous oxide by boiling in the potash; the fluid remains blue and diffuses very quickly through the potash.

In order to distinguish if the reduction of the red cuprous oxide comes from grape sugar or dextrine, a section of the same plant in which the reaction has been produced should be laid in alcohol of 90 or 95 per cent. Since according to Payen dextrine is itself insoluble in alcohol of 84 per cent, it cannot be extracted from the cells by a considerable stronger alcohol. The section then containing dextrine should show the red reaction when treated as described, even after lying for several hours in the alcohol. If, on the contrary, the cells of a thin section contain grape sugar an alcohol of 90 to 95 per cent will dissolve it, and after ten or twenty-four hours, as Payen shows, that alcohol of 95 per cent will dissolve grape sugar itself. If then a section before treatment with alcohol reduces with cuprous oxide, and after that shows no precipitate of red grains, one may conclude, according to Sachs, that the reduction of cuprous oxide is due to grape sugar and not to dextrine.

With the inexperienced, an error may occur both in the reaction for grape sugar and dextrine and for cane sugar. The outer walls of wood cells, vessels, etc., as is well known, are colored orange-yellow to reddish by this treatment, parenchyma cells and very young wood cells, etc., blue (see p. 326). These colors to the unaided eye might awaken a suspicion that in the former case grape sugar or dextrine reaction had taken place, and in the latter the reaction of cane sugar; but a sufficient magnification would give the correct information.

IV. VEGETABLE MUCILAGE.

Literature. Vogel und Schleiden, Ueber d. Amyloid, eine neue Pflanzensubstanz (Poggendorff's Annalen, Bd. XLVI, 1839, p. 327).—Kützing, Grundz. d. philos. Bot., Leipzig, 1852, Bd. I, p. 159, *ff.*—Cramer, Botan. Beiträge, Zürich, 1855, p. 1, *ff.*—Karsten, Ueber d. Entstehung des Harzes, Wachses, Gummis und Schleimes durch d. assimil. Thätigkeit d. Zellmembranen (Botan. Zeitg., 1857, p. 313, *ff.*).—Frank, Ueber d. anat. Bedeut. und die Entstehung der vegetab. Schleime (Pringsheim's Jahrb., Bd. V, 1866, pp. 161–200).—Hanstein, Ueber d. Organe der Harz- und Schleimabsonderung in den Laubknospen (Bot. Zeitg., 1868, p. 697, *ff.*).—Barciano, Ueber d. Blütentwicklung der Onagraceen (Schenk und Lürssen's Mitth. aus d. Geb. d. Bot., Bd. II, p. 85).—Kirchner und Tollens, Untersuchungen über d. Pflanzenschleime (Ann. Chem. Pharm., Bd. CLXXV, p. 205).—Behrens, Die Nectarien d. Blüten (Flora, 1879, p. 440, *ff.*)—Szyszylowicz, Korallina jako odczynnik mikrochemiczny w histyjologii roslinéj (Osobne odbicie z Rozpran Akad. Umiej w Krakowie, Bd. X, 1882).—For the rest see the treatises cited on p. 327.

This widely distributed vegetable substance which likewise bears the formula $C_6H_{10}O_5$ or $C_{12}H_{20}O_{10}$ has by no means been so thoroughly studied as is necessary for an exact knowledge of it. We have already explained (p. 328, *ff.*) that it is frequently produced by a metamorphosis of the cell membranes; that there are even various transformations of essential cellu-

lose into mucilage which are accounted for with the greatest difficulty. Other mucilage may doubtless be indebted to starch for its origin (mucilage of the orchid bulbs); still others present a mixture of mucilage and gum. Generally, it is a matter of no small difficulty to keep the gums and mucilaginous substances separate. In a general way it may be said that all mucilage gives a blue-violet and sometimes a yellow color with iodine, and with oxalic-nitric acid; that the gums give no color with iodine but are transformed into mucic acid by nitric acid.

In the present condition of theories concerning the nature of vegetable mucilage it would be hazardous to undertake a classification of them; the following on that account may be looked upon as somewhat imperfect. Of the mucilages we distinguish:

1. *Characteristic Mucilage.* In water it is either insoluble, soluble or simply swells. Alcohol precipitates it from an aqueous solution. It colors blue or violet with iodine, or iodine and sulphuric acid; with Hanstein's aniline mixture red or reddish (if at all colored), and yields oxalic acid when treated with nitric acid.

(*a*) *Mucilage from Cellulose* (*e. g.*, roots of *Symphytum*).

(*b*) *Mucilage from Starch* (*e. g.*, Orchis bulbs).

2. *Amyloid.* Mostly soluble in water; with iodine it gives a yellow, yellow red and also a blue color. From an aqueous solution alcohol precipitates a jelly-like substance which takes a blue color from iodine. With Hanstein's aniline it gives a beautiful red or scarlet-red color. Perhaps nearly related to 1 *b*.

3. *Lichenin* (lichen starch). Swells but does not dissolve in water; precipitates by alcohol; iodine alone colors it yellow, green or blue.

4. *Gum Mucilage.* Swells or dissolves in water, giving with iodine no color or yellowish to reddish; indifferent to most anilines; treated with nitric acid it yields mucic acid, or oxalic with mucic acid.[96]

[96] Giraud (according to Husemann, Pflanzenstoffe, Bd. I, p. 130) divides plant mucilage into two kinds, those producing pectin (gum tragacanth) and those not. The latter are separated into those which are changed into an insoluble form by acids and those not precipitated by acids.

1. *Characteristic Mucilage.*

This is very widely distributed through the vegetable kingdom, being formed, for example, in the swelling secondary layers in the parenchyma of the cotyledons of many plants, and in many muciparous roots, as of *Symphytum*, *Orchis*, etc. Whether the amyloid which was first detected by Schleiden and Vogel in the cotyledon of *Lupinus*, *Tropæolum*, etc., is to be ascribed to it, or represents a type of mucilage of its own, must remain temporarily doubtful. I have recently expressly demonstrated that in the neighborhood of muciparous cells, often in them, crystals or bundles of crystal-needles of calcium oxalate are found.[97] I then expressed the opinion that they might stand in some relation to the mucilage existing there though one might not be able to say how. The probability of this interdependence is considerably augmented by the fact that plant mucilage is chemically transformed into oxalic acid by an oxydizing medium, as, for example, nitric acid. Frank[98] observed some time ago that in the formation of the orchis mucilage the oxalic needles found in that vicinity slowly diminished.

Many mucilages belonging to this group are colored, with iodine alone or with iodine and sulphuric acid, a blue or violet, giving the reaction of cellulose.[99] With others this is not the case, as this treatment produces a yellow or yellowish color.[100] Hanstein's aniline mixture gives to most of the mucilages a full, red color which sometimes has a tinge of purple. Successive treatment with creosote, zinc chloride and aniline will cause a reddening of the mucilage (Barcianu). (The effect of aniline on mucilage has been but little studied.)

Quite recently Szyszylowicz has designated coralline as a

[97] Behrens, Die Nectarien der Blüten (Flora, 1879, p. 450).
[98] Frank in Pringsheim's Jahrb., Bd. V, p. 181.
[99] Kützing, Grundz. d. philos. Bot., Bd. I, p. 195.—Frank, *l. c.*, pp. 168, 181.
[100] Frank, *l. c.*, pp 163, 165, 167.—Hanstein in Bot. Zeit., 1868, p. 700, etc.

preferable reagent for vegetable mucilage, and indeed it is possible by means of this reagent to distinguish the mucilage which comes from starch from that which is produced from cellulose.

Coralline or rosalic acid, a red powder with greenish, metallic luster, dissolves in very dilute alcohol and in alkali salts, but not in water, with a beautiful coral-red color.[101] Szyszylowicz prepared it from carbolic acid by the action of sulphuric acid in the presence of oxalic acid. It then contained some aniline mixed with it. He dissolved it in sodium carbonate (probably because an alcoholic solution would produce the precipitation of the mucilage). The solution is purple-red and unchangeable in the light.

According to Szyszylowicz this reagent gives to starch mucilage a permanent red, the color not being destroyed even by prolonged boiling in alcohol; protoplasm and the cell walls, however, remain perfectly colorless. Cellulose mucilage is likewise colored, but the color may be removed by cold and especially by hot alcohol. Many colored preparations of starch mucilage may be preserved, preferably in Canada balsam; others on the contrary cannot.

2. *Amyloid.*

This is also, as has been stated, very like typical mucilage. It is often very sensitive to the action of iodine reagents. With Hanstein's aniline mixture it yields a red or scarlet-red color.[102] Hanstein appears to suppose that there are several kinds of amyloids, on what grounds it is not very clear to me. Whether what Hanstein calls "collagene" is to be included in this or rather among the gum mucilages is also doubtful. He once declared collagene[103] to be of those cellulose-like bodies which are finally transformed into mucilage, simply by absorbing water, not observing if it chemically stood

[101] This substance is to be had everywhere, since in recent times it has come into general use in the chemical laboratory as an important test in volumetric determinations in the place of litmus.

[102] Hanstein, *l. c.*, a. v. O.

[103] Hanstein, *l. c.*, p. 700, note.

nearer to this or another amyloid; afterwards he mentioned[104] that gum mucilage was produced essentially out of wall-building amyloid substances. Finally, he was never able to bring the collagene layer to the red reaction with aniline, as it is always possible to do with amyloid.

3. *Lichnin or Lichen Starch.*

This appears to stand very near to starch. It occurs in many lichens (*Cetraria*, *Cladonia Ramalina*) and in marine algæ (*Delesseria*) in the form of small grains or as a jelly-like mass. Lichnin swells but does not dissolve in water. Alcohol precipitates it unchanged, but caustic alkalies dissolve it. With iodine alone it colors yellow, grey or blue. It is dissolved by chlor-iodide of zinc and cuprammonia.

4. *Gum Mucilage.*

We designate by this expression several slimy substances which in part may be a mixture of gum and mucilage, and in part simple substances standing between the gums and mucilages. Although they often occur they are but little known. To this group belong, for example, the mucilage of many fuci, the mucilage of the quince, the mucilage of the seeds of flax, of *Plantago psyllium* and *P. lanceolata* and the althea mucilage. Here also perhaps the collagene of Hanstein may be best placed. With nitric acid it is decomposed into mucic and oxalic acid, while the true gums give only mucic acid. According to Szyszylowicz the gums do not thoroughly stain with coralline; they do color with it more or less, the intensity and permanency of the color depending upon the relations of the two substances.

V. GUM. (ARABIN, BASSORIN.)

Literatu e. Meyen, Pflanzenpathologie, 1841, pp. 229–255.—Mohl, Unters. über d. Entstehungsweise des Traganthgummi (Bot. Zeitg., 1857, p. 33, *ff.*)—Karsten, Ueber d. Entstehung

[104] Hanstein, *l. c.*, p. 774.

d. Harzes, Wachses, Gummis, etc. (*id.* 1857, p. 313, *ff.*).— Hofmeister, Ueber d. zu Gallerte aufquellenden Zellen, etc. (Ber. d. sächs. Gesellsch. d. Wiss. zu Leipzig, Bd. X, 1858, pp. 18–36).— Trécul, Production de la gomme chez le Cérisier, le Prunier, l'Amandier, l'Abricotier et la Pêcher (Procès-verbaux des séances de la soc. philomat. pend. l'année, 1862, voir aussi Journal de l'Institut, 1862, p. 241).— Wigand, Ueber d. Desorganisation d. Pflanzenzelle, insbes. über d. physiol. Bedeutung von Gummi und Harz (Pringsheim's Jahrb., Bd. III, 1863, pp. 115–186).—Kraus, Ueber den Bau d. Cycadeenfiedern (*id.* Bd. IV, 1865, pp. 328–329).— Frank, Ueber d. anat. Bedeut. u. d. Entstehung der veget. Schleime (*id.* Bd. V, 1866, pp. 161–200).—Müller, Unters. über die Vertheilung d. Harze, äther. Oele, Gummi und Gummiharze, etc. (*id.* Bd. V, 1866, p. 397, *ff.*).—Hofmeister, Lehre v. d. Pflzelle., Leipzig, 1867, p. 234, *ff.*—Hanstein, Ueber d. Organe der Harz- und Schleimabsonderung in den Laubknospen. (Botan. Zeitg., 1868, p. 697, *ff.*).—Sorauer in Landwirthschaftl. Versuchsst., 1872, p. 454.—Prillieux, Etude sur la format. de la gomme dans les arbres fruitiers (Ann. des sc. nat. Botanique, 6e sér. t. I, 1875, pp. 176–200).—Szyszylowicz, Koral lina jako odczynnik mikrochemiczny w histyjologii roslinnéj (Osobne odbicie z. Rozpran Akad. Umiej w Krakowie t. X).[105]

The numerous extremely heterogeneous substances which are united under this name, and to which may be ascribed the formula $C_6H_{10}O_5$ or multiples of it, occur in plants as mucilaginous, colorless, yellow or brown masses which are either soluble in water and are then called *Arabin* or *Cerasin* (gum arabic, cherry gum), or are insoluble in water and are then named *Bassorin* or *Adragantin* (gum tragacanth). The previously named pectinaceous substances probably also belong with these as, according to the opinion of many chemists, pectose is nothing else than changed arabin. On the other hand it appears that many species of bassorin and adragantin, for example gum tragacanth, are strongly impregnated with pectose. The gums, like the mucilages, have been but little studied heretofore, so that the proposal of the

[105] Compare also the literature cited on p. 327 and p. 343, *f.*, the latter in reference to pectin metamorphosis.

chemists to unite together temporarily in one category all pectinaceous substances, mucilages and gums, and to designate them "jelly-forming carbohydrates," has much in its favor.

Preliminarily it may be said only that the gums in a restricted sense are distinguished from the mucilages in that they give no reaction with iodine reagents and that when treated with nitric acid they do not change as those do into oxalic acid but into mucic acid. Recently it has been stated by Szyszylowicz that in opposition to true mucilage they do not color with coralline.

They occur either alone or mixed with mucilage (mucilage gum) or they form a more or less intimate mixture with the resins (resin gums). Of the occurrence of the latter kind, some very beautiful examples have been described by Hanstein[106] in the young leaf buds.

The gum masses are either pure secretions such as are poured forth in a pathological condition of the plant, as, for example, the cherry gum, or that peculiar formation of gum in the wood of the orange tree which in Italy is known as the "Mal della gomma."[107] But in other cases the formation of the gum is a normal process. Then it will commonly be poured out into receptacles, gum conduits.

The production of gum tragacanth out of parts of the cell wall was first studied by v. Mohl and rightly explained (see p. 329). Karsten regarded all kinds of gums as the product of the cell wall. Trecul and Frank agree with this but Wigand made various objections. Trecul allows that sometimes the contents of the vessel in part participate in the formation of the gums and describes reservoirs for the formed gum. Frank held that the pouring out of gum was always a symptom of disease, the cause of which lay in the heaping up of plastic matter in a certain place whereby the equilibrium of the interior of the plant is disturbed.

Prillieux agrees with Trecul and finds that gum is formed both in gum conduits and in the vessels the latter having in the

[106] Hanstein in Bot. Zeitg., 1868, p. 704, *ff.*

[107] Novellis, Il male della gomma degli agrumi (L'Agricoltura Meridionale, Portici 1879; Cf. des Verf. Besprechung in Bot. Centralbl. Bd. II, 1880, p. 469 *f.*)

cherry tree both pitted and spiral thickenings. It is sometimes produced from starch. According to Hofmeister[108] it is an improper expression to speak of the formation of gum by the disorganization of the cell wall, since it existed before the destruction of the walls as cell contents and, together with the transformed walls, forms the secretion. Gum substances appear never to occur in the cell sap.

A. Arabin and Cerasin. Cherry gum is colored yellow with iodine and chlor-iodide of zinc, brownish with potash, yellow, or in earlier stages, a lively violet with muriatic acid (Prillieux) and dissolves in water.[109]

The gum in the conduits of the pinnæ of the *Cycadeæ* is not colored by chlor-iodide of zinc and forms flocculent masses with alcohol.[110] Coralline leaves the gum uncolored. All walls attacked by gummosis frequently become violet with aniline, according to Hanstein.

B. Bassorin. Adragantin. According to Mohl, gum tragacanth is not colored by iodine or chlor-iodide of zinc. According to Prillieux[111] there may be noticed in it layers of cell walls which color blue violet with chlor-iodide of zinc. According to this author it consists of a mixture of cellulose and of a mucilaginous substance which is interposed between the very thin layers of the cellulose.

C. The Mucilage of Flax seed is a gummy mucilage which behaves like bassorin, swelling, but not dissolving, in water and not coloring blue with iodine and sulphuric acid, at most only yellow. It is insoluble in cuprammonia not even swelling in it. The mucilage of *Plantago psyllium* is similar but dissolves with ease in cuprammonia, but does not color blue with iodine and sulphuric acid. The gummy mucilage of the root of *Althæa officinalis* behaves in the same way.

[108] Hofmeister, Pflanzenzelle, p. 234.

[109] According to Prillieux (Ann. Sc. Nat. *l. c.* p. 182 *f.*) there is found in the vessels of the cherry wood a kind of gum (Cerason) different from Cerasin which is not soluble in water and is colored rose red with iodine and sulphuric acid. It is very much like the so-called Eugelacin of Kützing found in many algæ. It is colored violet with muriatic acid.

[110] Kraus in Pringsheim's Jahrb., Bd. IV, p. 329.

[111] Prillieux, *l. c.* p. 181 *f.*

[112] Frank, *l. c.*, p. 161–167.

VI. INULIN.

Literature. Rose, Ueber eine eigenth. vegetab. substanz (Gehlen's neues Journ. d. Chem., Bd. III, 1804, pp. 217–219) —Payen, Extrait d'un mém. lu a l'Institut sur une nouv. subst. trouvée dans les tuberc. des dahlias (Journ. de Pharm., t. IX, 1823, pp. 383–392).—Payen, Observ. sur l'analyse des tuberc. de l'Helianthus tuberosus (Ann. de Chim. et de Phys., t. XXVI, 1824, pp. 98–106).—Meyen, Neues Syst. d. Pfl.-Phys., Bd. II, 1838, pp. 281–285.—Payen, complément d'un mémoire, etc. (Ann. des sc. nat. 2e sér., t. XIV, Botanique, 1840, p. 85, *ff.*). —Cramer, Ueber d. Verhalten des Kupferoxydammoniaks zur Pflanzenzellmembran, zu Stärke, Inulin, etc. (Vierteljahrsschr. d. nat. Ges. Zurich, Bd. III, 1858, pp. 1–22).—Mohl, Unters. des Pflanzengew. mit Hilfe des polar. Lichtes (Botan. Zeitg., 1858, p. 1, *ff.*).—Hartig, Entwicklungsgesch. d. Pflkeims, Lpz., 1858, p. 117.—Schacht, Ueber d. Inulin (Sitzungsber. niederrh. Gesellsch. zu Bonn, 1863, pp. 174–177).—Sachs (*id.*, pp. 177–180).—Sachs, Ueber d. Sphärokrystalle des Inulins (*id.*, 1864, pp. 9-11).—Sachs, Ueber d. Sphärokrystalle des Inulins und dessen Mikrosk. Nachweisung in den Zellen (Botan. Zeitg. 1864, p. 25, *ff.*).—Prantl, Das Inulin. Ein Beitrag zur Pflanzenphysiologie, Munchen, 1870.—Dragendorf, Materialien zu einer Monographie des Inulins, Petersburg, 1870.—Kraus, Beobacht. über d. Vorkommen des Inulins (Sitzungsber. naturf. Gesellsch. zu Halle 27, Feb., 1875.)[113]

Inulin, called also Alantin, Helenin, Dahlin, has the formula $C_6H_{10}O_5$ or, according to Kiliani, $C_{36}H_{62}O_{31}$. It occurs in the underground organs of the Compositeæ (*Taraxacum officinale*, *Helianthus tuberosus*, *Georgina variabilis*, *Inula Helenium*, *Eupatorium cannabium*, etc.) in *Campanula rapunculoides*, in some other phanerogams and in numerous cryptogams (*Acetabularia mediterranea*, *Elaphomyces granulatus*, etc). In the first named plants it always occurs in the parenchyma cells (Sachs,

[113] Prantl gives a full list of the literature, also of the chemical, *l. c.* pp. 67–72; of the chemical after 1870, see Husemann, Pflanzenstoffe, Bd. I, 1882, p. 137.

Berg, Prantl),[114] and indeed in the cell sap,[115] in the form of a concentrated solution which is pretty strongly refractive. It is almost insoluble in cold water, but dissolves in water warmed to from 50° to 55°.[116] Inulin is insoluble in alcohol, ether, glycerine, fatty or essential oils, the first precipitating it from an aqueous solution. On this is founded the one only method of microscopically testing it. Strong acids dissolve inulin after it first becomes transparent, likewise potash lye and zinc chloride solution. Cuprammonia also dissolves inulin, the solution beginning, according to Cramer, not on the surface but in the center of the spherical crystal. If the inulin be slowly crystallized from a solution, it forms the spherical crystals of a most highly characteristic structure, which grow by a process of superposition.[117] Iodine reagents do not color inulin, as would be self-evident, since it is incapable of swelling.[118] If a microscopic section containing precipitated inulin be boiled for some minutes in water having a trace of muriatic acid, a considerable quantity of cuprous oxide may be reduced inside the cells by a copper sulphate and potash process described on pp. 293 and 365. This cannot be done with a fresh section, only with one which has previously been boiled in acidulated water. By this process the inulin is transformed into glycose (levulose).[119] Heated on the platinum slip, inulin develops a vapor which strongly smells like burning sugar.[120]

Method of microscopical examination (Sachs).[121] From tissue containing inulin should be cut sections more than one cell layer thick and then covered with a large drop of alcohol. This produces a milky precipitate. After some minutes, however, the preparation clarifies itself by the formation of spherical crystals. Now by dipping it in water the small granules disappear and the spherical crystals alone remain behind, the irlam-

[114] Prantl, *l. c.*, p. 39.

[115] Mohl, Bot. Zeitg., 1858, p. 17.—Schacht, Sitzungsber. Bohn, 1863, p. 175.—Sachs, *id.*, p. 177.

[116] Sachs, Botan. Zeitg., 1864, p. 78.

[117] Nägeli in Sitzungsber. K. Bayer, Acad. d. Wiss., München, 1862, Bd. II, p. 314, *ff.*—Sachs, *l. c.*, p. 80.

[118] Prantl, *l. c.*, p. 30.

[119] Sachs, Sitzungsber., Bonn, 1863, p. 178, 1864, p. 10.

[120] Sachs, *l. c.*, p. 78.

[121] Sachs, *l. c.*, p. 85.

inated structure becoming more distinct (Fig. 133).[122] The crystals become much larger, often breaking through the tissue, if we put large organs containing inulin for a long time, days and weeks, in alcohol and glycerine and then prepare the sections from them. Also by allowing such organs to dry, one can detect inulin in them in the form of spherical crystals, after the preparation of the sections. According to Sachs the external appearance of the inulin crystals is sufficient to identify them as such, but, according to Prantl, a more exact testing of their qualities as given above is indispensable.

FIG. 133.

VII. GRAPE SUGAR, GLYCOSE.

Literature. Sachs, Ueber einige neue mikrosk.-chem. Reactionsmethoden (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. XXXVI, 1859, p. 5, *ff.*).— Sachs, Mikrochem. Unters. (Flora, 1862, p. 289, *ff.*).—Sachs, Ueber d. Stoffe, welche d. Material z. Aufbau d. Zellwand liefern (Pringsheim's Jahrb., Bd. III, 1863, p. 183, *ff.*).

Grape sugar, also called glycose or starch sugar, $C_6 H_{12} O_6$, very frequently occurs in plant tissues and almost without exception in aqueous solution, and mostly in connection with solutions of cane, or other kinds of sugar. It arises as a transformation product of other carbo-hydrates chiefly indeed of starch (starch sugar) and in this form appears to travel through the interior of plants.

The single method for the testing of grape sugar, as is that of cane sugar and dextrine, is based on the reaction of copper sulphate and potash discovered by Trommer and modified by Fehling. It was introduced into microscopy by Sachs.

Impregnate the tissues containing the glycose with copper sulphate solution and add thereto cold potash lye in excess so as to produce a beautiful blue fluid. Directly, if cold, sooner

[122] Nach Sachs, *l. c.*, Taf. II, fig. 5.

by boiling, there appears a copious display of reduced copper oxide which is colored a beautiful brick red. Under the microscope the precipitate appears like that of dextrine; the grains, however, are larger and collected in many large flocculent masses which is not the case with dextrine. The manipulation to be followed in this reaction is the same as that described on page 365, for dextrine; for this reason it should be remarked here, that, as in the case of dextrine, the section to be tested for grape sugar should have a thickness greater than that of a single layer of cells, else will the fluid cell contents escape and the desired reaction will not take place.

In place of a pure copper sulphate solution one containing a tartaric acid salt may be employed (Fehling's solution). The latter is prepared by dissolving one part by weight of copper sulphate, and five parts of potassium sodium tartrate in eight parts of water afterwards keeping the solution in the dark.

VIII. CANE SUGAR, SACCHAROSE.

Literature. The same as in the grape sugar.

As is the grape sugar so is the cane sugar or saccharose, $C_{12} H_{22} O_{11}$, a widely distributed plant substance which presents itself in the cells likewise as a clear solution.

The method for microscopical testing as given by Sachs is the same as that for grape sugar. Treat a section containing cane sugar with Trommer's or Fehling's solution and then add potash lye and there will appear a beautiful blue coloring, but no reduction of copper oxide takes place even after a short boiling. The appearance of the blue coloring in the cold liquid is quite characteristic and is indeed definitely perceptible even with quite thin sections.

The reduction of copper oxide shows always the presence of grape sugar or of dextrine, but with cane sugar a reduction never occurs.

Concerning testing for a mixture of these substances with each other or with albuminous matter see Sachs in the proceedings of the Imperial Academy of Sciences, Vienna, Vol. XXXVI, p. 10, f.

IX. ALBUMINOUS MATTER. PROTEIDS.

Among nitrogenous substances in plants, albuminous or proteid matter holds the chief place. It is lacking in no living cell and all vital processes are intimately connected with it. Chemically it is characterized by its components of carbon, oxygen, nitrogen and hydrogen, with a less but constant quantity of sulphur. As to the rest a satisfactory chemical formula has not yet been made. Perhaps this is in general impossible, since the albuminous substances met with in plants are probably not individuals in the chemical sense. Such a formula has been long desired for protoplasm for instance. The recently published chemical analyses of the protoplasm of *Æthalium septicum*[123] by Rodewald show it to consist of a large number of different organic and inorganic chemical elements.

Albuminous substances occur in cell contents; they are solid or plastic-viscous, often indistinguishable from a fluid. They are insoluble in absolute alcohol, soluble or insoluble in water. They are mostly colorless, rarely yellowish, and still more rarely reddish or bluish.

As universal qualities of albuminous substances, which make their recognition easily possible under the microscope, are the following: that they take a yellow or dark yellow, or brown color from any weak solution of iodine, the color being more intense than that of the surrounding iodine solution; that they enter into a dark-yellow combination with nitric acid which was invested by Mulder with the name of xanthroproteid acid; that they take a beautiful violet color with copper sulphate and potassium; that they give a beautiful rose-red with Millon's reagent; that they become red with a solution of sugar and the aid of sulphuric acid; that they are unchanged by Hanstein's aniline solution. Dead but not living albuminous matter generally may be stained with coloring substances, as carmine solutions, hæmatoxylin, etc.

[123] Reinke, Ueber d. Zusammensetz. d. Protopl. v. *Æthal. sept.* Göttingen 1880.—Reinke u. Rodewald, Studien über d. Protoplasma, I, Die Chem. Zusammensetz. des Protopl. v. *Æthal. sept.* (Unters. aus d. Bot. Laborat. d. Univ. Göttingen, Heft II, 1881, pp. 1–75).

A chemical classification of proteid substances has indeed frequently been attempted, but heretofore with no very satisfactory results. Ritthausen,[124] one of the best of those who know these substances, divides them into albumin (plant albumin), plant casein (legumin, conglutin and glutencasein) and paste-like proteids (gliadin, mucedin and glutenfibrin). Pfeffer[125] provisionally holds every chemical classification to be insufficient and with A. Mayer[126] classifies albuminous bodies as reserve and functional proteid substances. This classification is the foundation of the following representation.

I. Reserve Proteid Substances.

(Proteid grains, Aleuron, Gluten Meal.)

Literature. Hartig, Ueber das Klebermehl (Bot. Zeit., 1855, p. 881).—Hartig, Weitere Mittheil. d. Klebermehl (Aleuron) betreffend (*id.*, 1856, p. 257, *ff.*).—Hartig, Entwicklungsgesch. d. Pflanzenkeims, Lpz., 1858, pp. 108–134.—v. Holle, Beiträge z. näheren Kenntniss d. Proteinkörner im Samen der Gewächse (Neues Jahrb. f. Pharm., Bd. X, 1858, pp. 1–24).—Cohn, Ueber Proteinkrystalle in den Kartoffeln (37, Jahresber. d. schles. Gesellsch. f. vaterl. Cultur, 1858, pp. 72–82).—Trécul, Des formations vésiculaires dans les cellules végét. (Ann. des sc. nat. 4e sér., t. X, 1858, p. 20, *ff.*).—Maschke, Ueber d. Bau u. d. Bestandtheile der Kleberbläschen in Bertholletia, deren Entwicklung in Ricinus (Bot. Zeitg., 1859, p. 409, *ff.*).—Radlkofer, Ueber Krystalle proteinartiger Körper pflanzlichen u. thierischen Ursprungs, Leipz., 1859.—Nägeli, Ueber d. Krystallähnl. Proteinkörper u. ihre Verscheidenh. v. wahren Krystallen (Sitzungsber. d. K. Acad. d. Wiss. z. München, Jahrg., 1862, Bd. II, pp. 120–154).—Cramer, Das Rhodospermin, ein krystalloidischer, quellbarer Körper im Zellinhalt verschiedener Florideen (Vierteljahrsschr. d. naturforsch. Gesellsch. in Zürich, Jahrg., VII, 1862, pp. 350–365).—Nägeli, Pflanzenphysiol. Unters.—Sachs, Zur Keimungs-

[124] Husemann, Pflanzenstoffe, 1882, Bd. I. p. 233, *ff.*
[125] Pfeffer in Pringsheim's Jahrb., Bd. VIII, p. 491.
[126] A. Mayer, Lehrb. d. Agricul. Chem., 1877, p. 191.

gesch. d. Dattel (Botan. Zeitg., 1862, p. 241, *ff.*).—Sachs, Ueber d. Keimung des Samens v. *Allium cepa* (*id.*, 1863, p. 57, *ff.*).—Gris, Recherches anatom. et physiol. sur la germination (Ann. des sc. nat. 5e sér. t. II, 1864, pp. 1–123).—Cohn, Beitr. z. Physiol. d. Phycochromaceen und Florideen (Schultze's Archiv f. mikrosk. Anat., Bd. III, 1867, pp. 1–60.)—Dippel, D. Mikrosk., Bd. II, 1869, p. 29, *ff.*—Klein, Ueber d. Krystalloïde einiger Florideen (Flora, 1871, pp. 161–169.—Klein, Zur Kentniss des Pilobolus (6 Geformte Inhaltskörper) (Pringsheim's Jahrb., Bd. VIII, 1872, p. 337, f.).—Pfeffer, Unters. über d. Proteinkörner u. d. Bedeutung des Asparagins beim Keimen der Samen (*id.*, pp. 419–574).—Sachs, Lehrbuch, pp. 53–59.—Prillieux, Sur la coloration et le verdissement du Neottia Nidus-avis (Ann. des sc. nat. 5e sér., t. XIX, 1874, pp. 108–118).—Van Tieghem, Nouvelles recherches sur les Mucorinées (Format. et rôle des cristalloides de mucorine) (*id.*, 6e sér., t. I, 1875, pp. 24–32).—Schimper, Unters. über d. Proteinkrystalle d. Pfl. Strassb., 1879.—Klein, *Pinguicula alpina* als insectenfr. Pfl. u. in anatomischer Bezeihung (Cohn's Beiträg. z. Biol. d. Pfl., Bd. III, 1880, pp. 163–184).—Klein, Neuere Daten über d. Krystalloïde Meeresalgen (Flora, 1880, p. 65, *ff.*).—Vines, On the Chemical compos. of Aleuron-grains (Proceedings of the Royal Soc. of London, Vol. XXX, 1880, p. 387, *ff.*).

Proteid grains (v. Holle, Pfeffer), also called aleuron or gluten meal (Hartig), were discovered in the year 1855 by Theodore Hartig and thoroughly investigated first, by him, and later by Sachs, but principally by Pfeffer. They occur in the endosperm or in the parenchyma of the cotyledons of the seeds of a number of plants,[127] and indeed, often in company with starch, embedded in the fatty protoplasmic substance of the cells. They are formed in the last stages of the ripening of the seeds and change again at the beginning of the sprouting. They are represented by very small, small or large granules. Sometimes there are found in the same cell many small granules and one great proteid grain (Solitär, Hartig). They consist altogether of

[127] Compare, concerning their occurrence, Hartig, Entwicklungsgeschichte d. Pflanzkeims, pp. 120–124 and the above cited treatises of Pfeffer.

proteid matter, perhaps being mingled with a small quantity of other substances.

The statement of Sachs that they consist in part of fatty matter has been, recently, refuted by Pfeffer. The mass of the granules is either amorphous, or a part of the proteid matter assumes a crystal-like nature (proteid grains with crystalloids). Still others contain inorganic substances. The latter are either true crystals of calcium oxalate or crystalline roundish bodies, the so-called globoids which consist of calcium and magnesium phosphates. We will, therefore, describe in their turn: (*a*) amorphic proteid grains, (*b*) proteid grains with crystalloids, (*c*) proteid grains inclosing inorganic matter.

A. Amorphic Proteid Grains.

All amorphic proteid grains are insoluble in absolute alcohol and ether (both must be absolutely anhydrous), in chloroform and benzole, in fatty and essential oils. But benzole especially dissolves the fat of the surrounding fundamental substance, and in oily seeds the proteid grains appear more distinctly after its application (for the rest see below). If water be gradually added to a preparation lying in alcohol many proteid grains show a concentric lamination. This appearance often occurs (*Pæonia*, endosperm) when alcohol containing a little sulphuric acid is employed and the section examined in water.[128]

FIG. 134.

The lamination, however, appears only in the peripheral part of the grain, the center remaining amorphic (Fig. 134, after Pfeffer). Many proteid grains are quite soluble in water, others partly, still others not at all. All are soluble in water which contains a trace of caustic potash, likewise in acids and alkalies, many in glycerine and sugar solution (in this often slowly). Those grains which are soluble in water must be examined in absolute alcohol or oil; their presence is best demonstrated by iodine in glycerine, iodine with a little potassium iodide dissolved in glycerine, see page 286.

[128] Pfeffer, *l. c.*, p. 499.

Pfeffer has given a method by which proteid grains which are soluble in water may be transformed into an insoluble modification. He says,[129] "I make use of the property of proteid matter to become insoluble in water by corrosive sublimate and be transformed into an achlorate mercurial combination. In order to get this without disorganizing the proteid grain, I digest the section of the seed for at least twelve hours in a small flask with a solution of simple mercuric chloride in absolute alcohol, of the concentration of which it is not necessary to be very particular, though I find in most cases about a 2 per cent solution to be best. Then wash the section in alcohol and carry it into water in which it is now quite insoluble. It is not recommended to wash out the section very carefully in alcohol. I may also remark that in taking the section from the fluid no needle or scalpel of steel should be used, since iron makes a precipitate with metallic mercury which would contaminate the surface of the section coming in contact with it. On this account one should use a glass rod or most conveniently a needle or small scalpel made of platinum, which latter one can make for himself for this purpose by cutting a piece of thin platinum and fitting it into a glass tube by melting the glass. The proteid grains thus prepared will indeed swell in water but will return to their original volume after a time by treatment with alcohol." They are, however, soluble in dilute acids and alkalies and give the same reactions as the fresh proteid grains. The process especially fits them for the study of the effects of acid reagents upon them.

By this method of making the proteid grains insoluble, proof is at the same time afforded that gums, pectinaceous substances or cane sugar, occur, if at all, only in very small quantities in proteid grains. These substances enter into no insoluble combinations with corrosive sublimate, so that they would be dissolved out after this process when subject to the action of water and the granule would change in form and mass which it does not in fact.[130]

If proteid grains, which are or have been made insoluble in

[129] Pfeffer, *l. c.*, p. 141.
[130] Pfeffer, *l. c.*, p. 442.

water, be boiled with water or treated with alcohol or ether, they will coagulate and are then scarcely soluble in dilute alkalies and acids at common temperatures, but gradually dissolve in concentrated alkali.

Every proteid grain is surrounded with a delicate envelope. If by the means indicated the fundamental substance of the proteid grain be dissolved this envelope remains behind and the application of reagents shows that it consists of proteid substances.

The principal microscopical reactions of proteid grains are the following. Iodine will in all solutions give a brown or yellow-brown color (use a neutral solution where the granules have not been modified by coagulation); numerous other coloring substances are also absorbed by the grains. Pfeffer[131] used principally for this purpose the aniline blues dissolved in water, solutions which remain unchanged for a long time. The proteid grains absorb therewith, in small quantity, a much more striking color than with cochineal extract. Nitric acid and potash give the yellow color proceeding from xanthoproteid acid; sugar and sulphuric acid a rose red (p. 299); Millon's reagent a brick red (p. 296). The latter, according to Pfeffer, is not worthy of much commendation, likewise copper sulphate and potassium should be rejected, which Nägeli and Schwendener[132] have very highly recommended for this purpose. Cuprammonia does not dissolve the proteid granules.

All amorphic proteid grains are not doubly refractive and therefore are not illuminated on a dark field in the polarizing apparatus (Caspary, Hartig).[133]

A few words may be added concerning the protoplasmic foundation of the cells which contain proteid grains.[134] It is protoplasm whose water is replaced by oil, the latter occurring in the form of small or large drops. Benzole or ether easily dissolves it away leaving behind the fine, granular, often very scanty fundamental mass. The oily contents of the fundamental substance is made very beautifully visible by means of alcanna red

[131] Pfeffer, *l. c.*, p. 444.
[132] Nägeli and Schwendener, Mikrosk., p. 530.
[133] Hartig, Entwicklungsgesch. d. Pflk., p. 109.
[134] Pfeffer, *l. c.*, pp. 478–485.

(Pfeffer). Use a deeply colored, about 70 or 80 per cent, alcoholic extract of alcanna root (see p. 310). Move the seed-section back and forth in this a few times, wash it off in weak alcohol, and put it immediately in strong glycerine for examination. The coloring matter dissolved in alcohol does not penetrate into the proteid granules in the short space of time in which it comes in contact with them. But the alcoholic solution of the coloring matter adhering to the section, is quickly dissolved by the oil in the fundamental mass with which it comes into such intimate contact.. By its distribution through the fundamental mass, sections of very oily seeds are soon colored a beautiful blood red. When the fundamental substance itself is very deeply colored the proteid grains appear to be isolated,[135] quite colorless. If the oil is very easily dissolved in alcohol, as in the seeds of *Ricinus*, the alcanna tincture should be diluted with an equal quantity of glycerine.

If the cells containing proteid grains be treated with potash solution or potash water after the oil has been removed with benzole or ether, the grains will be dissolved away and the fundamental mass, as well as the envelopes of the granules, will be left as a delicate network, which looks often not unlike parenchyma tissues.[136] Iodine solutions color it brown-yellow, Millon's reagent red. It consists of albuminous matter. The nucleus lies shrunken up within.

B. Proteid Grains with Crystalloids.[137]

A great number of proteid grains contain formed proteid matter, which appears in the form of crystal-like bodies. Hartig called them "white granules," Nägeli named them crystalloids. They are surrounded by the envelope of the granule (Fig. 135, A, B,), which may moreover often be almost entirely wanting. Sometimes several crystalloids are found united in one grain (Fig. 135, C, D, after Pfeffer). In oil and alcohol the crystalloids are not usually visible in consequence of the like

[135] Pfeffer, *l. c.*, Taf. XXXVIII, Fig. 21.
[136] Pfeffer, *l. c.*, Taf. XXXVIII, Fig. 2.
[137] Pfeffer, *l. c.*, pp. 450–464.

refracting power of the crystalloids and the envelope. In order to make the former visible the grains must be put into water. This either dissolves the surrounding mass of the envelope or makes it swell thus causing the crystalloids to appear. The enveloping mass has the same qualities as the substance of the proteid grains without crystalloids.

Of the characteristics of the crystalloids we mention the following: The crystal systems are not very thoroughly known. The crystalloids of *Bertholletia* should, according to Nägeli, be clinorhombic; others belong, according to Hartig, to the tesseral and rhombic systems. Sorauer found, besides these, four-sided columns. All crystalloids, although but slightly, are double refractive.[138] According to Radlkofer this latter characteristic is increased by coagulation. The angles of the crystalloids are very inconstant; the addition of water changes them 2 or 3 degrees, swelling about 15 or 16 degrees. According to Nägeli the crystalloids, like the starch grains, consist of two distinct substances (see p. 360), according to Maschke, of casein and a little albumen; according to Pfeffer both views are groundless.

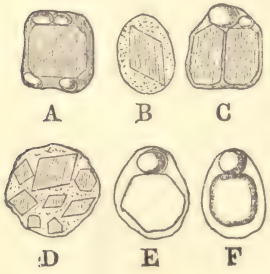

FIG. 135.

All crystalloids are insoluble in water; they can, however, be freed from the fundamental mass by water alone or with the addition of sodium phosphate. They are insoluble likewise in absolute alcohol but, on the contrary, are soluble in glycerine containing potash, in dilute potash as well as in muriatic and acetic acid. After solution every crystalloid leaves behind a little envelope (Fig. 135, E and F, after Pfeffer). The crystalloid should be dissolved by concentrated glycerine with a trace of potash. The envelope gives the same reaction as that of the grain itself. By boiling, the crystalloids are transformed into the insoluble modification of proteid matter. They are then insoluble in dilute potash but will swell considerably in it.

*

* *

[138] Maschke in Bot. Zeitg., 1859, Tab. XV, Figs. 95, 96, 98, 99, 101, 102.

Proteid Crystalloids without an Inclosing Mass.

In the proteid grains containing crystalloids the inclosing mass is often quantitatively considerable, but sometimes the crystalloids are inclosed only by a very thin layer. These proteid grains are closely related to those crystalloids which are quite uncovered. In the latter case the proteid crystalloids lie free in the cells or in the plasma. A sharply defined boundary between the free lying crystalloids and those inclosed in an integumentary mass can scarcely be drawn. Free proteid crystalloids frequently occur in resting seeds as well as elsewhere, in phanerogams as well as in cryptogams. We limit ourselves here to the presentation of some carefully studied cases.

1. *Crystalloids of the Potato tuber* (Cohn). In the cuticular layer of the potato tuber occur numerous cubic crystalloids measuring from 0.007 to 0.013 mm. on a side. Cohn[139] gives the following reactions for these forms which are feebly double refractive. Iodine colors them yellow to deep golden brown, sugar and sulphuric acid a peach blow red, Millon's reagent a brick red, carmine with the least possible ammonia a deep red, cochineal extract with water and a subsequent addition of acetic acid (Maschke) intense burning red. They are slowly soluble in glycerine, soluble in ammonia from outward toward the center, in acetic acid from the center outward, and in dilute potassium lye (but not in concentrated; in this they swell and are stained yellow). Sulphuric, nitric and muriatic acid dissolve most of the crystalloids or make them swell up to a globular drop. By boiling in water the crystalloids in general remain unchanged but become more easily visible and show a laminated structure.

2. *Crystalloids in Bertholletia excelsa* (Hartig, Maschke, Radlkofer, Nägeli). The crystalloids in the endosperm of the Para nut have been frequently described, but the statements concerning the reactions produced on them by the several naturalists are wide apart, which Nägeli claims is chiefly caused by using the reagents in very different degrees of concentration by which

[139] Cohn, 58 Ber. d. Schles. Gesellsch. f. vaterl. Cultur, 1859, pp. 74–77.

the tested crystalloids would present themselves in a very different manner. Hartig held the crystalloids to be hexagonal, Maschke tesseral; Nägeli finally made it very probable that they are clinorhombic (Fig. 136, A, B, after Nägeli). According to Nägeli they do not dissolve in water. Hartig had stated that they were soluble in water, Radlkofer that it took but slight hold of them. By boiling in water they coagulate and are not then soluble in weak alkali (Radlkofer). Alcohol and ether do not alter them even when boiling hot. According to Nägeli

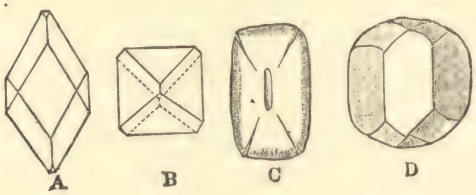

FIG. 136.

glycerine does not dissolve them, only increases their volume. Radlkofer says that they dissolve in it very slowly. Acetic acid will not dissolve them even in the presence of glycerine. Very weak acids do not change the crystalloids, stronger dissolve them gradually or quickly; first, however, making them swell up (Fig. 136, C, D). In nitric acid they become round and full of vacuoles and in time yellow. Ammonia dissolves them with less swelling, likewise weak potash, while the concentrated does not dissolve them, only rounds them up. Iodine colors them brown or yellow brown, Millon's reagent red; coloring matter is energetically absorbed by them.[140]

3. *Crystalloids in Pilobolus* (Klein, Van Tieghem). In the fruit bearers of *Pilobolus* occur likewise many small crystalloids which were investigated in *Pilobolus crystallinus* by Klein, and in *P. roridus* by Van Tieghem (Fig. 137, after Van Tieghem and Klein). They are colorless and appear to be octahedral or quadratic pyramidal, and have sides

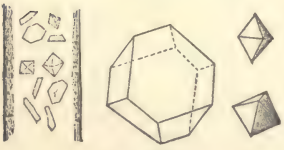

FIG. 137.

[140] Nach Nägeli, Sitzungsber. Bayer. Acad., 1862, Bd. II, pp. 128–137; Radlkofer, Krystalle proteinartiger Körper, pp. 65–69.

which are not quite plane. Potash either swells or dissolves them. Iodine colors them yellow or brown. If the iodine is dissolved in alcohol they are shrunken at the same time; alcohol alone contracts them. Sulphuric acid alone colors them a rose red. It colors the plasma of *Pilobolus* likewise the same. The long continued action of concentrated nitric acid colors them a pale yellow.[141]

4. *Crystalloids in Floridia.* (*Rhodospermin.*) (Cramer, Klein, Cohn). Crystalloids of proteid bodies have been observed in different algæ, as, for example, *Bornetia secundiflora* Thuret, *Callithammon caudatum* Ag., *C. seminudum* Ag., *Griffithsia barbata* Ag., *Gr. Nepolitana* Näg., *Gongocerus pellucidum* Ktzg., and designated by Cramer *Rhodospermin*, since they have commonly been stained by the red coloring matter of the algæ and are rose red. Cohn and Klein demonstrated afterwards that the crystalloids in the living plants are mostly colorless. The crystalloids are either simply refractive and belong then to the hexagonal system (needles or plates of from 0.004 to 0.050 mm. long), or they are double refractive, clinorhombic (octahedral-like forms of from 0.01 to 0.04 mm. long). Cramer distinguished therefore hexagonal and clinorhombic Rhodospermin. (*a*) *Hexagonal Rhodospermin:* insoluble in water, and absolute alcohol (cold or boiling), glycerine and acetic acid, cold muriatic acid (boiling slowly destroys it), sulphuric acid likewise and nitric acid. Nitric acid alone does not color it, but does with the addition of ammonia. It is insoluble in dilute and concentrated potash lye, ammonia and cuprammonia. These substances, however, cause it to swell and in boiling potash lye it is slowly destroyed. Iodine colors it first gold-yellow then brown-yellow; ammoniacal carmine solution colors it red but not essentially different from the surrounding solution; carmine solution with the addition of cooking salt, on the contrary, an intense red. Sugar with sulphuric acid gives no reaction. (*b*) *Clinorhombic Rhodospermin:* Iodine, nitric acid with ammonia behave as with the hexagonal Rhodospermin. In potash and

[141] Klein in Pringsheim's Jahrb., Bd. VIII, p. 337. *ff.*, und 376.—Van Tieghem in Ann. des sc. nat. 6e Sér., t. I, p. 25, *f.*

ammonia it swells but contracts again with nitric, sulphuric or muriatic acid. Millon's reagent colors it a brownish yellow.[142]

5. *Colored Crystalloids* in the pulp of the fruit of *Solanum Americanum* Mill (Nägeli). These occur in the form of thin plates and rhombs (Fig. 138, A, after Nägeli) which are frequently united (B), belong to the rhombic system and possess an intense violet color. The crystalloids consist partly of albu-

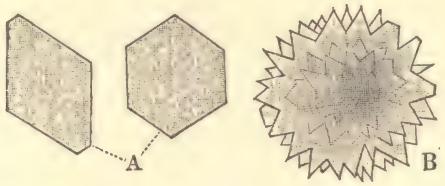

FIG. 138.

minous substances which are permeated with the violet coloring matter. Water does not alter them but if it be slightly sour or alkaline the tone of the color is changed. Alcohol bleaches them from the inside outward and dissolves most of them, likewise ether. Iodine colors them a brown-yellow. Very weak acids color the crystalloids bright red, strong acids bleach and disintegrate them into separate pieces and finally dissolve them. Potash and boiling water behave alike; essential oils and chloroform are without effect upon the dry crystalloids.[143]

C. Proteid grains inclosing Inorganic Substances.

As already briefly mentioned there are proteid grains which contain within themselves inorganic substances. These are either globoids, spheroids (Fig. 139, A, after Pfeffer), or crystals (B). The former are a combination of magnesia and lime with a little phosphoric acid, the latter consists of calcium acetate. Frequently spheroids and globoids occur with crystal-

[142] According to Cramer, *l. c.*, Cohn in Schultze's Archiv, Bd. III, p. 24, *f.* Klein in Flora, 1871, pp. 161–169.

[143] Nägeli, Sitzungsber. d. Bayer. Acad., 1862, Bd. II, pp. 147–154; Vgl. auch Nägeli in Pflanzenphysiol. Unters., Bd. I, p. 6.

loids in the same proteid grains, but more seldom globoids with crystals.

(*a*) *Proteid grains with globoids.* The globoids (Kranzkörper of Hartig) have a roundish or cluster-like form (Fig. 139, A). They occur in almost every seed that contains reserve proteid matter. The largest (Vitis) attain a diameter of 0.01 mm. In order to investigate globoids (as crystals) first remove the oil from the section of the seed and then dissolve away the proteid substance with water or very dilute potash (Pfeffer). The globoids are singly refractive, insoluble in cold and boiling water and alcohol, soluble in all mineral acids and acetic acid (without effervescence). They take no color from iodine or aniline blue. They gradually dissolve in an ammoniacal chlorine-ammonia solution, likewise in alcohol which contains a little sulphuric or oxalic acid. In the latter case after a considerable time one may find in place of the globoids tiny needle crystals of calcium and magnesium oxalate. Concentrated potassium and ammonia dissolve a substance out of the globoids from the outside inward. They then appear as a finely granulated, feebly refractive mass with a cuticular layer which may be stained like proteid matter with iodine and aniline blue. No swelling is produced by the action of the potash.

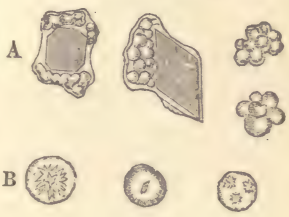

FIG. 139.

(*b*) *Proteid grains with crystals.* The crystals occur as clinorhombic plates, etc., or as a cluster of crystals grown together. They are insoluble in water, and acetic acid; calcined, the residuum dissolves in the latter with effervescence. They are insoluble in not too concentrated potash lye. (For further reactions see under section XII, Inorganic Vegetable Elements.) If the crystals are carefully dissolved in muriatic acid, there remains behind a delicate skin consisting of proteid matter, also in the middle something like a nucleus is found. Both can be recognized with certainty when a little iodine is added to the dilute muriatic acid used in the solution.

2. Functional Proteid Matter.
(Protoplasm and Cell nucleus.)

Literature. V. Mohl, Einige Bemerk. über d. Bau d. veget. Zelle (Bot. Ztg., 1844, p. 273, *ff.*).—V. Mohl, Verm. Schr. Tübing., 1845, a. v. O.—V. Mohl, D. Veget. Zelle, Brschwg., 1851, p. 198, *ff.*—Schacht, D. Pflanzenzelle, Leipzig, 1852, a. v. O.—Hartig, Ueber d. Verf. b. Behandl. d. Zellkerns mit Farbstoffen (Bot. Zeitg., 1854, p. 877, *ff.*).—v. Mohl, D. Primordialschlauch (Bot. Zeitg., 1855, p. 689, *ff.*).—Schacht, Lehrb. d. Anat. u. Physiol. d. Gew., 1856, a. v. O.—Hartig, Entwicklungsgeschicte d. Pflkeims, Lpz., 1858, a. v. O.—Maschke, Pigmentlösung als Reagenz bei mikrosk. physiol. Unters. (Botan. Zeitg., 1859, p. 21, *ff.*).—Radlkofer, Ueber Krystalle proteinartiger Körper, Lpz., 1859, p. 1, *ff.*—Sachs, Ueber einige neue mikrosk.-chem. Reactionsmeth. (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. XXXVI, 1859, p. 9, *ff.*).—De Bary, Ueber d. Bau u. d. Wesen der Zelle (Flora, 1862, pp. 243–251).—Sachs, Mikrochem. Unters. (Flora, 1862, p. 297, *ff.*).—Schultze, Ueber d. Bau d. Nasenschleimhaut (Abh. d. naturf. Gesellsch. zu Halle, Bd. VII, 1863, p. 92).—Sachs, Zur Keimungsgesch. d. Gräser (Bot. Zeitg., 1862, p. 145, *ff.*).—Sachs, Zur Keimungsgesch. d. Dattel (*id.*, p. 241, *ff.*).—Schultze, D. Protoplasma d. Rhizopoden u. d. Pflanzenzellen, Lpz., 1863, p. 39, *ff.*).—Cienkowsky, Zur Entwicklungsgesch. d. Myxomyceten (Pringsheim's Jahrb., Bd. III, 1863, p. 325, *ff.*).—Cienkowsky, D. Plasmodium (*id.*, p. 400, *ff.*).—Sachs, Beiträge z. Physiolog. d. Chlorophylls (Flora, 1863, p. 193, *ff.*).—Sachs, Ueber d. Keimung d. Sämens von *Allium cepa* (Bot. Zeitg., 1863, p. 57, *ff.*).—Sachs, Ueber d. Stoffe, welche d. Material z. Aufbau d. Zellhäute liefern (Pringsheim's Jahrbuch, Bd. III, 1863, p. 185, *ff.*).—De Bary, D. Mycetozoen, Leipz., 1864, p. 41, *ff.*—Kühne, Unters. über d. Protoplasma, Leipz., 1864.—Sachs, Handbuch d. Experimentalphysiol. d. Pfl., Leipz., 1865, p. 309, *ff.*—De Bary, Morph. u. Physiol. d. Pilze, Flechten u. Myxomyc., Leipz., 1866, p. 103, *f.*—Nägeli u. Schwen-

dener, Mikrosk., p. 529, *ff.*—Hanstein, Ueber d. Organe d. Harz- u. Schleimabs. an d. Laubknospen (Botan. Zeitg., 1868, p. 697, *ff.*).—Dippel, Mikrosk., Bd. II, Brschwg., 1869, pp. 9–18. — Schröder, Beitr. z. Kenntn. der Frühjahrsperiode des Ahorn (Pringsheim's Jahrb., Bd. VII, 1869, pp. 283, 314, 325). — Sachs, Lehrb., p. 39, *ff.*—Strasburger, Studien über Protoplasma. Jena, 1876.—Tangl, D. Protoplasma d. Erbse (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXVI, 1877, Decemberheft, Bd. LXXVIII, 1878, Juniheft.) —Treub, Quelques rech. sur le rôle du noyau dans la divis. des cellules végét., Amsterd., 1878.—Behrens, D. Nect. d. Blüten (Flora, 1879, a. v. O.) — Schmitz, Unters. über Structur d. Protopl. u. d. Zellkerne d. Pflzellen (Sitzungsber. d. niederrh. Gesellsch. zu Bonn, 1880, p. 159, *ff.*)—Strasburger, Zellbildung u. Zelltheilung, Jena, 1880, a. v. O.—Johow, Unters. über d. Zellkern der höheren Monokot. Bonn, 1880.—Hanstein, D. Protopl. als Träger d. pflanzl. u. thier Lebensverricht. Heidelbg., 1880.—Reinke, Studien über Protoplasma, Berlin, 1881. —Tangl, Ueber offene Communu. zwischen d. Zellen d. Endosp. einiger Samen (Pringsheim's Jahrb., Bd. XII, 1881, p. 170, *ff.*).—Detmer, D. Wesen d. Stoffwechselprocesse im veg. Organismus (*id.*, p. 253, *ff.*). Poulsen, Botan. Mikrochem., p. 52, *f.* (Trans. p. 63)[144] [E. Pfitzer in Bericht Deutsch. Botan. Gesellsch. I (1883), pp. 44–77.]

Functional proteid substances, protoplasm and cell nucleus are found in all living cells, and on account of their constant occurrence are also generally known. Functional proteid substance either fills the cell and has no constant form as a whole (protoplasm), or it has a form and is localized (lying in the protoplasm) and is enclosed in a delicate cuticular membrane (cell nucleus). Protoplasm is not always enclosed in the cellulose walls of surrounding cells; it may also exist in a living form by itself (Amœba, Plasmodia, Myxomyceta, swarm spores, etc.) It frequently exhibits (free or in cells) characteristic appearances of motion. It is seldom represented by a

[144] A perfect list of the literature concerning this subject is really impossible. In the present list only those treatises are quoted which describe microscopical methods of reaction. All others, for instance those which treat of the appearances of the movement of protoplasm, are not referred to.

horny, hard mass as in resting seeds. In most cases it is permeated by a greater or less quantity of water and then is plastic, soft and often very like a fluid. In it are almost always small or very small granules (oil drops) by which it gets a granulated appearance. The protoplasmic body is commonly surrounded outwardly by a solid hyaline ungranulated layer (cuticular layer). Protoplasm is composed chemically, first of all, of the (frequently prevailing) albuminous substances, also of a great number of other combinations (Sachs, Reinke and Rodewald); and lastly, of a small quantity of inorganic incombustible substances.

There are often found, temporarily or otherwise, in the protoplasm, other substances which are afterwards employed either for building the wall of the cell (cellulose builder, Sachs), or which are separated out as substances of secretion (metaplasm, Hanstein). The nucleus, which, as has been established by the recent investigations of Strasburger, Hanstein, Treub, Schultz and others, plays an important part in cell division, consists of several elements, concerning which one must consult the authors named.

All reactions to be hereafter referred to show both in the protoplasm and the nucleus, since in both albuminous substances predominate. Both will be described separately, care being taken that in treating of the nucleus, the methods already given with reference to protoplasm be not repeated.

A. Protoplasm, Epiplasm, Metaplasm.

1. *Protoplasm in the narrower sense.* Substances which absorb water, as absolute alcohol, concentrated glycerine, solution of common salt, absorb the water from protoplasm and cause it to shrink up or contract. It thus draws itself away from the cell wall and commonly assumes an irregular outline. Absolute alcohol, osmic acid, solution of picric acid kill protoplasm and cause it to stiffen. The more quickly this stiffening or hardening takes place, as, for example, in boiling alcohol, the more perfectly is the original structure preserved (Strasburger, see p. 178). A solution of common salt does not kill the shrunken protoplasm. A cell so treated is "plasmolized."

Plasma contracted by means of alcohol appears to be far less soluble in acids and dilute alkalies than when fresh (v. Mohl).

A characteristic quality of dead protoplasm is its ability to absorb a large number of coloring substances. Living protoplasm does not possess this power as already proved by Hartig, who grew *Algæ*, *Lemma*, *Chara*, *Hydrocharis* in carmine solution. The growth was not particularly hindered by the coloring matter, but the protoplasm and nucleus absorbed not the least trace of the pigment.[145] Furthermore the nucleus has the power of absorbing the coloring matter in a much higher degree than the protoplasm. Grenacher's carmine solution (p. 308) and most of the other carmine solutions as well as the extract of cochineal (see p. 305) can be commended. The resistant enveloping layer of the protoplasm behaves quite negatively towards most coloring substances.[146] Hanstein's aniline solution will be taken up by unchanged protoplasm as a blue-violet. Iodine in the familiar solutions (potassium iodide of iodine, chlor-iodide of zinc, glycerine iodine, and iodine and sulphuric acid), will give a yellow or brown-toned color. The brown shades are the most common and in many cases are very dark.

The alkalies behave toward protoplasm differently according to their degree of concentration, those most in use being potash and soda lyes whose action is much like that of the rest. Concentrated solution of potash leaves protoplasm entirely unchanged, neither dissolving nor swelling it. M. Schultze[147] therefore recommends strong potash lye as a mounting medium for protoplasmic preparations. Dilute potash solutions on the contrary make protoplasm first transparent and then soon perfectly dissolves it. Concentrated ammonia fluid clears it up very soon and dissolves it, though sometimes not perfectly till after several hours.

Concentrated mineral acids, for example, concentrated sulphuric acid, have the greatest dissolving power. This acid does not commonly color protoplasm, but if the protoplasm be anhydrous it becomes rose-red to brown. Sulphuric acid with con-

[145] Hartig in Bot. Zeitg., 1854, pp. 576, 877.
[146] Tangl in Pringsheim's Jahrb., Bd. XII, p. 174.
[147] M. Schultze in Abhandl. d. naturf. Gesellsch. z. Halle, Bd. VII, p. 92, *f.*

centrated solution of sugar colors every kind of protoplasm rose-red, and moreover this reagent is quite sensitive. First lay the preparation in the sugar solution, then put on a cover-glass and let the acid flow in from the edge. Phosphoric acid changes protoplasm but little; acetic acid makes it opaque. Nitric acid, used warm or cold, colors it yellow or brown with the formation of xanthoproteid acid; by adding potassium or ammonia there will be formed the related xanthoproteid salt which is distinguished by its positive, mostly brown, color.

Millon's reagent colors protoplasm brick-red, still it is on the whole not very sensitive. Indol with sulphuric acid colors it a feeble rose-red if at all (Niggl).

Copper sulphate with potash (method p. 365) colors all albuminous substances violet, which color will not be changed by continued boiling. By reflected light it is uniformly a dark violet; by transmitted it plays more into the wine-red.[148]

The Plasmodium of the Myxomycetæ[149] becomes rose-red by sugar and sulphuric acid, and Millon's reagent with iodine yellow. Alcohol and nitric acid cause coagulation; in acetic acid the substance becomes colorless and transparent. It liquefies in dilute potash solution, likewise in potassium carbonate which first often somewhat shrinks it. Alcohol, glycerine, chlorate of zinc, iodine and dilute chromic acid leave the marginal layer at first unchanged, but on the other hand quickly contracts the remainder of the plasmodium.

2. *Epiplasm.* De Bary[150] designates by this name the protoplasmic residuum in the spore sacs of the *Ascomycetæ* which still remains after the spores are formed. It is more strongly refractive than the common protoplasm, has a characteristic, homogeneous sparkling appearance, and is very sensitive toward iodine solutions, the most dilute of which colors it a beautiful red to a violet-brown.

3. *Metaplasm.* Under this designation of Hanstein[151] we are to understand protoplasm in which are contained considerable quantities of carbo-hydrates, the most important

[148] Sachs in Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. XXXVI, p. 9.
[149] De Bary, d. Mycetozöen, p. 41, *f.*
[150] De Bary, Morphology und Physiol. d. Pilz. Flectin und Myxomyc., p. 103, *f.*
[151] Hanstein in Bot. Zeit., 1868, p. 710.

of which are the amyloid-like substances, which sooner or later will be separated from it to be applied to the construction of cell walls or as secretions. In many organs of secretion the albuminous substances of the metaplasm are so far thrust into the background that it is with the greatest difficulty they are detected by the reagents commonly used.[152] In the remainder the albuminous substances are detectable by the previously described methods of reaction. Metaplasm behaves characteristically toward Hanstein's aniline mixture as has already been shown. It is not colored by it, like common protoplasm, blue-violet[153], but scarlet-red, this color being more fuchsin-red when tannic acid is present in it (see below).

B. Cell Nucleus.

As has been previously indicated, the cell nucleus gives the same reactions as other proteid substances. For a long time a considerable number of histological reagents have been employed to make the nucleus itself more clear and bring out all the fine structural relations which had heretofore been indistinct. These reagents are again in use, and especially since the epoch-making investigations of Strasburger. The unusual activity in the study of the nucleus has brought to light a great number of these reagents. We can, therefore, in the following, name but a few of the more important, and for the rest may refer to those works which treat of these matters, and which every phytotomist who would be conversant with the questions of the day must study.

Hartig first attempted to stain the nucleus with a carmine solution in water which had absorbed ammonia from the air. He added some drops of metallic quicksilver or iodine solution to it in order to make it keep.[154] He found, furthermore,[155] that the nucleus with nitrate of silver was colored almost black under the influence of light, and that when it was laid first in a dilute solution of ferrocyanide of potassium, then carefully washed out and treated with a dilute solution of ferric chloride it

[152] Behrens in Flora, 1879, p. 444, *ff.*
[153] Hanstein, *l. c.*, Taf. XI, Figs. 17, 23.
[154] Hartig in Bot. Zeitg., 1854, p. 877.
[155] Hartig, *l. c.*, p. 878.

was colored a deep blue. But if we apply Berlin blue direct, the nucleus becomes not blue but a pale, smutty, reddish color. If in addition to this we note that it was known that the nucleus became distinct in acetic acid, and thus was first made visible, but that by the use of concentrated acid it was made to swell, we have exhausted pretty nearly all the histological nucleus-reagents of the older authors.

In later times we aim at two things in the use of these reagents: (1) fixing the nucleus; (2) rendering it visible by staining. We will particularly consider both.

A. Fixing the Structure of the Nucleus.

The fixing (or "setting") is done by very dilute organic or inorganic acids. In almost all cases the fixing takes place very quickly so that the preparation need remain but a short time in the reagent. Acetic acid works very well and a solution of not higher than one per cent should be used. It then produces no shrinking of the nucleus but its stringy framework comes out very distinctly. By the use of stronger acid the swelling produced makes it again very soon quite indistinct. In place of the acetic one may use formic acid (Retzius). For other cases chromic acid gives excellent service in a $\frac{1}{6}$ to $\frac{1}{2}$ per cent solution, sometimes even as strong as a one per cent solution.[156] Picric acid may also be employed in different degrees of dilution. Nitric or picro-sulphuric acids are less worthy of commendation since they occasion a considerable shrinking, the preparation becoming much less beautiful than in chromic acid. On the other hand osmic acid is a very excellent fixing medium used in a one per cent solution.[157] It makes the structure of the nucleus very distinct but in many cases indeed causes it to swell. Strasburger used it, for example, in following out the division of the nucleus in the mother cell of the pollen. He emptied the pollen sack into a 3 per cent solution of sugar and added a drop of 1 per cent solution of osmic acid, when all the relations

[156] Strasburger, Zellbildung u. Zelltheilung, pp. 172, 173, etc.
[157] Strasburger, *l. c.*, p. 39 und anderwärts.

came out sharply after some minutes.[158] Fleisch[159] has proposed for a like purpose a mixture of chromic and osmic acid, which according to Fleming fixes it well enough but the structure appears pale and is stained with difficulty. But this objection according to Fleming[160] is obviated if one adds a little acetic acid to the mixture. Then a very beautiful staining with hematoxylin, picro-carmine, and gentiana is obtained. Fleming's mixture consists of chromic acid, 0.25 per cent, osmic acid 0.1 per cent and acetic acid 0.1 per cent in distilled water.

[Absolute alcohol fixes the protoplasm without contracting it. The section or the whole organ may be plunged into it. Strasburger by putting *Spirogyra orthospira* in absolute alcohol at different hours of the night succeeded in fixing the division of the nuclei of this alga in their various stages of development so they could be studied the next day very easily. The same observer also retarded the development of the nuclei till morning by placing the plant in a room without heat in November and so could watch the development by daylight and fix them at the most suitable moment. A. B. H.]

B. Staining the Nucleus.

By the use of these means the structure of the nucleus is fixed, that is, becomes distinct. We now proceed to stain it. This may be done with the aniline dyes (p. 299, *ff.*), hæmatoxylin (p. 304), cochineal extract (p. 305), or carmine solutions (which see). Those most worthy of commendation are the following. (The methods of preparing these staining media have already been given.)

1. *Staining with Borax-carmine* (Strasburger).[161] The section commonly needs to lie in the mixture, described on p. 307, but a short time. Concerning the examination and preservation of the specimen see that page.

[158] Strasburger, *l. c.*, p. 21. For a like purpose Hartig, earlier, treated pollen grains with carmine glycerine twelve to twenty-four hours (Bot. Unters. herausgeg. v. Karsten, 1866, Heft 3, p. 249).

[159] Fleisch in Arch., f. mikrosk. Anat., Bd. XVI, p. 300.

[160] Fleming, Zellsubstance, Kern und Zelltheilung, Lpz., 1882, p. 381.

[161] Strasburger, *l. c.*, p. 9.

2. *Staining with Beale's carmine* (Strasburger). Commended for filamentous algæ. For particular directions see p. 307.

3. *Staining with Acetic acid carmine* (Fleming). This fluid is suitable only for fresh sections, which sometimes become very beautiful in it.

4. *Staining with Picro-carmine* (Fleming, Treub). Alike commendable for animal and vegetable tissue. The section needs to lie in the fluid but a very short time. Mount in glycerine.

5. *Staining with Hæmatoxylin* (Frey, Strasburger, Fleming). Those sections which have been fixed in osmic acid and have been freshly washed are especially to be commended for this staining medium, as they then take up the coloring matter well. If they have lain in alcohol for a long time they color badly. Staining may be done in either a strong or dilute solution; in the latter case it will require from twenty-four to forty-eight hours. If the section becomes over-colored, alum water or dilute muriatic acid will clear it up; in the use of the latter the nucleus will be slightly swollen. Make use of the hæmatoxylin solution given by Frey or one of the new things recommended by Grenacher.[162]

6. *Staining with Picro-hæmatoxylin.*[163] This as I can testify from some experiments of my own is a very superior method and is described by Schmitz as follows. The section of the fresh plant is put in a concentrated solution of picric acid and remains for a shorter or longer time, even over night if necessary. In this picric acid solution the protoplasm immediately hardens. By longer continuance in the solution the plasmic part of the cells contracts a very little, but that is in many cases an advantage to the investigation, for by this means the cell membrane becomes much more transparent to the coloring matter of the plasma without itself becoming stained. As a coloring matter for the plasmic body I now almost always use hæmatoxylin in an aqueous solution without the addition of

[162] Prepare a saturated solution of crystallized hæmatoxylin in absolute alcohol and a like one of ammoniacal alum in water. Mix 4 cc. of the former with 150 cc. of the latter. Let it stand for a week in the light, filter and add 25 cc. of glycerine and 25 cc. of mythel alcohol. After all the free precipitate has settled the reagent is ready for use.

[163] Schmitz, in Sitzungsb. der niederrhein. Gesellsch. zu Bonn, 1880, p. 160.

alum. I lay the object in water, it having been freed from every trace of picric acid by repeated and careful washings, and add a small quantity of hæmatoxylin which has absorbed ammonia from the air and so is partly changed into hæmatein-ammonia. The coloring matter dissolves rapidly in pure water with a red color and gives a solution which gradually darkens and after some time decomposes. After remaining for some time (from one to several hours) in the solution, whose degree of concentration must be chosen, according to the special purpose in view, the object should be taken out and washed in water till the wash water remains quite colorless. Then the object will, according to the quantity of the coloring matter used and the length of time given to its effect (this must be tested for each case), be colored blue, in more or less intense shade, either the chromate bodies of the nucleus alone, or these and the rest of the substance of the nucleus, as also the thicker plasmic bodies, as for example the crystalloids, or the whole of the plasmic elements of the cell; but the whole cell membrane, starch grains, oil drops and crystals remain almost colorless. The color is best preserved when the preparation is mounted in glycerine, but one must be absolutely sure that not a trace of free acid remains in the specimen. The least particle of acid will infallibly destroy the color in time and very provokingly render the most excellent specimens useless.

7. **Staining with Methyl green.* (Strasburger, Fromann, Fleming.) Strasburger[164] uses a one per cent solution of acetic acid which he dilutes with methyl green (commended by Meyzel). In order to see the form of the nucleus of the pollen mother cell, he puts a young anther in the acetic methyl green solution and breaks it open by pressure. The outcoming contents are immediately fixed and the figure of the nucleus becomes at once beautifully stained by the methyl green. Alas! that a preparation so made should not keep.

8. *Staining with other Aniline coloring substances.* Among the many coloring substances here commended may be specially mentioned Saffranin, Dahlia, Gentiana violet, the latter with

[164] Strasburger, *l. c.*, p. 141.

acetic acid, as affording very beautiful colors. Aniline preparations are best preserved in dammar varnish or Canada balsam, but they shrink if they are for this purpose previously put in oil of cloves. Fleming[165], therefore, recommends mounting them in resinous turpentine oil after previously passing through dilute and then absolute alcohol with which one may gradually mix the turpentine oil.

[*Pfitzer's Reagent for simultaneous Staining and Hardening.* E. Pfitzer has reported a fluid which both hardens and stains vegetable protoplasm. It consists of the coloring matter, nigrosin, dissolved with picric acid in water or alcohol.]

[(*a*) To a concentrated solution of picric acid is added a small quantity of an aqueous solution of nigrosin. If the object to be studied contains much water some crystals of the acid should be added to maintain the strength of the liquid.]

[The deep olive-green fluid kills with great rapidity. After some hours' immersion of the object which is to be examined it may be transferred to alcohol, especially if it be desired to dissolve out the chlorophyll, or if the object has to be kept some time. By this means the denser masses of protoplasm are stained pale violet, the chromatophores darker, while the pyrenoid, nucleoli and other colored parts of the nucleus come out deeply stained; thin films of protoplasm and ordinary cellulose membranes are scarcely, if at all, stained; starch grains not at all. By washing the objects in *water* after staining instead of in spirits, a gray-blue color is obtained; transference to strong glycerine makes the color purer. The color comes out best however after washing in alcohol, treating with oil of cloves and mounting in one of the resins, dammar or Canada balsam].

[To avoid contraction the clove oil may be diluted with alcohol and allowed to concentrate upon the object by evaporation of the alcohol. The watery solution is especially adapted for rapidly killing and staining objects already under the microscope.]

[(*b*) Nigrosin and picric acid may also be used in solution in alcohol. The solid acid and nigrosin are left for some time in

[165] Fleming, *l. c.*, p. 384.

TABLE OF THE MOST IMPORTANT REACTIONS FOR THE FLUID CONTENTS OF THE CELLS.

		Iodine reagents.	Copper sulphate and potash.	Nitric acid.	Millon's reagent.	Hanstein's aniline.	Alcohol.	General appearance.	
1	Dextrine	—	Red precipitate	—	—	—	Unchanged	Homogeneous Fluids	1
2	Inulin	Uncolored	—	Dissolves	Uncolored	Uncolored	Precipitates spherical crystals		2
3	Grape sugar	—	Red precipitate	—	—	—	Unchanged		3
4	Cane sugar	—	Blue fluid	—	—	—	Unchanged		4
5	Vegetable mucilage	Blue, violet or yellow	?	Dissolves giving oxalic acid	—	Reddish or red, but not all	Becomes flocculent or jelly-like	Jelly-like Fluids	5
6	Gums	Uncolored	Blue flocculent precipitate	Dissolves giving mucic acid	—	Uncolored (rarely violet?)	Becomes flaky or jelly-like		6
7	Proteid substances	Yellow, brown, seldom red-brown	Violet	Brown	Brick red	Purple violet	Becomes coagulated or contracted	Granular plastic substances	7

absolute alcohol; by this solution the chromatophores and pyrenoids are less deeply stained; the colored contents of the nucleus very deeply so.]

[Quoted from Jour. Roy. Microscop. Soc., Vol. III, No. III, pp. 445–6. A. B. H.].

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Under the headings III, IV, V, VI, VII, VIII and IX we have treated those frequently-occurring substances, the contents of cells, which appear to be colorless fluids or very like such. As with the solid framework of the cell (see p. 356) so with these we also tabulate the principal reagents used in their identification (p. 403).

X. CHLOROPHYLL (LEAF-GREEN).

Literature. v. Mohl, Vermischte Sch. Tüb., 1845, p. 352, *ff.* (Unters. über d. Anat. Verhalten d. Chlorophylls).—v. Mohl, Ueber d. Bau d. Chloroph. (Bot. Zeitg., 1855, p. 89, *ff.*).—Böhm, Beitr. z. näheren Kenntn. d. Chl. (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. XXII, 1857, pp. 479–512). Gris, Recherch. microsc. sur la chl. (Ann. des sc. nat., 4e sér., t. VII, 1857, pp. 179–219).—Hartig, Entwicklungsgeschichte d. Pflankeims., Leipzig, 1858, pp. 79–82.—Morren, Diss. sur les feuilles vertes et colorées, 1858.—Sachs, Ueber d. Ergebnisse einige neu. Unters. über d. Chl. (Flora, 1862, p. 129, *ff.*). —Sachs, Ueber d. Einfl. d. Lichtes auf d. Bild. d. Amylums in d. Chlkörnern (Bot. Zeitg., 1862, p. 364, *ff.*).—Sachs, Beitr. z. Physiol. d. Chl. (Flora, 1863, p. 193, *ff.*).—Sachs, Ueber d. Auflös. u. Wiederbild. d. Amylums in d. Chlk. (Bot. Zeitg., 1864, p. 289, *ff.*).—Sachs, Handbuch d. Experimentalphys. d. Pfl., Lpz., 1865, pp. 309, *ff.*; 313, *ff.* — (Nägeli u. Schwendener, Mikros., Lpz., 1867, p. 496). — Micheli, Quelq. obser. sur la matière col. de la chl. (Arch. d. sc. d. la bibliothe. univ. de Genève, Mai, 1867).—Dippel, Mikrosk., Bd. II, p. 32, *ff.*—Kraus, G., Einige Beob. über d. Einfl. d. Licht. u. d. Wärme auf die Entsteh. d. Stärkeerzeugung im Chlorophyll (Pringsheim's Jahrb., Bd. VII, p. 511, *ff.*).—

Wiesner, Chl. in Neottia Nidus-avis betr. (Bot. Zeitg., 1871, p. 619).—Kraus, G., Z. Kenntn. d. Chlorophyllfarbst. u. ihrer Verwandten., Stuttg., 1872.—Wiesner, Unters. über d. Farbstoffe einiger für chlorophyllfrei gehaltenen Phanerog. (Pringsheim's Jahrb., Bd. VIII, 1872, p. 575, *ff.*).—Kraus, G., Einige Bemerk. über d. Erscheinung d. Sommerdürre unserer in Baum- und Strauchblätter (Bot. Zeitg., 1873, p. 401, *ff.*). —Briosi, Ueber normale Bild. v. fettart. Subst. im Chlrophyll. (*id.*, p. 529, *ff.*).—Drude, D. Biol. v. Neottia Nidus-avis u. Monotropa hyp. Göttingen, 1873.—Kraus, G., Ueber d. Ursache d. Färbung d. Epidermis vegetat. Organe d. Pfl. (Flora, 1873, p. 316, *ff.*).—Treub, Z. Chlorophyllfrage (*id.*, 1874, p. 55, *f.*)—Wiesner, Bemerk, über d. angelb. Bestandth. des Chl. (*id.*, p. 278, *ff.*).—Prillieux, sur la color. et le verdiss. du Neottia Nidus-avis (Ann. des sc. nat., 5e sér., t. XIX, 1874, p. 109, *ff.*).—Batalin, Ueber d. Zerst. Chloroph. in d. leb. Organen (Bot. Zeitg., 1874, p. 433, *ff.*).—Wiesner, Vorl. Mitth. über d. Einfl. d. Lichtes auf Entsteh. u. Zerstör. d. Chl. (*id.*, p. 116, *ff.*).—Sachs, Lehrb., p. 47, *ff.*—Wiesner, Welche Strahlen des Lichtes zerlegen bei Sauerstoffzutritt d. Chloroph.? (Poggendorff's Annalen, Bd. CLII, 1874, p. 496, *ff.*).—Pringsheim, Ueber d. Absorptionsspectra d. Chlorophyllfarbst. (Monatsber. K. Acad. Berlin, 1874, pp. 628–659). —Pringsheim, Ueber d. natürl. Chlorophyllmodificationen, etc. (*id.*, 1875, pp. 745–759).—Askenasy, Ueber d. Zerstör. d. Chl. leb. Pfl. durch d. Licht. (Bot. Zeitg., 1875, p. 457, *ff.*).—Haberlandt, Ueber d. Einfl. d. Frostes auf d. Chlk. (Oesterr. Bot. Zeitschr., 1876, p. 249, *ff.*).—Haberlandt, Ueber d. Entsteh. d. Chl. in d. Keimbl. v. *Phaseolus* (Bot. Zeitg., 1877, p. 361, *ff.*).—Sachsse, Chem. u. Phys. d. Farbstoffe, Kohlehydrate, etc., Lpz., 1877.—Dippel, Einige Bemerk. über d. Gemength. d. Chlorophylls, etc. (Flora, 1878, p. 17, *ff.*). Hoppe-Seyler, Ueber d. Chloroph. d. Pfl. (Bot. Zeitg., 1879, p. 815, *ff.*).—Pringsheim, Ueber d. Lichtwirk. u. Chlorophyllfunction in d. Pfl. (Bot. Zeitg., 1879, p. 789, *ff.*).—Pringsheim, Ueber Lichtwirk. u. Chlfunction in d. Pfl. (Monatsber. K. Acad. Berlin, 1879, Juli, 17 pp.).—Pringsheim, Ueber d. Hypochlorin u. d. Bedingungen s. Entst. in d. Pfl. (*id*, 1879,

Nov., 21 pp.).—Flahault, Sur la présence de la mat. verte dans les org. actuellem. soustraits à l'infl. de la lum. (Bull. de la Soc. bot. de France, t. XXVI, p. 249, *ff.*).—Pringsheim, Z. Kritik d. bisherig. Grundlagen d. Assimilationstheorie d. Pfl. (Monatsber. d. K. Acad. Berlin, 1881, pp. 117–135).—Pringsheim, Ueber d. primäre Wirk. d. Lichtes auf d. Veget. (*id.*, pp. 504–535).—Pringsheim, Ueber Lichtwirk. u. Chlfunct. in d. Pfl. (Pringsheim's Jahrb., Bd. XII, 1881, p. 288, *ff.*).—Hansen, Gesch. d. Assimilationstheorie u. Chlorophyllfunction., Lpz., 1882 (auch Arb. Bot. Inst. Würzbg., Bd. II, Heft. 4).—Tschirch, Unters. über d. Chloroph. (Botan. Centralbl., Bd. XI, 1882, p. 107, *ff.*).—Wiesner, Bemerk. über d. Natur d. Hypochlorins (*id.*, Bd. X, 1882, p. 260, *ff.*).—Pringsheim, Ueber Chlorophyllfunct. u. Lichtwirk. in d. Pfl. (Pringsheim's Jahrb., Bd. XIII, Heft 3; 116 pp.).—Schimper, Ueber d. Gestalten d. Stärkebildner und Farbkörper (Botan. Centralb., Bd. XII, 1882, p. 175, *ff.*).—Meyer, Ueber Chlorophyllk., Stärkbildner u. Farbk. (*id.*, p. 314, *ff.*).

Spectroscopic behavior of Chlorophyll. Brewster, on the color of natural bodies (Transact. Roy. Soc. of Edinburgh, t. XII, 1834, p. 538, *ff.*) Angström, Ueber d. grüne Farbe d. Pfl. (Poggendorf's Annalen, Bd. XCIII, 1854, p. 475, *ff.*), also (Ofversigt af. K. Ventesk. Acad. Förhandl, 1853, p. 246, *ff.*).—Stockes, Ueber d. Veränd. d. Brechbark. d. Lichtes (Poggendorff's Ann., Ergänzungsbd. IV, 1854, pp. 217–228).—Harting, Ueber d. Absorptionsvermögen d. reinen und unreinen Chlorophyll fur die Strahlen der Sonne (*id.*, Bd. XCVI, 1855, p. 543, *ff.*).—Askenasy, Beitr. z. Kenntn. d. Chl. u. einiger dass. begleit. Farbst. (Bot. Zeitg., 1867, p. 225, *ff.*).—Sorby. On a definite method of qualit. analysis of anim. and vegetable coloring matters by means of the spectro-microscope (Proceed. of the Roy. Soc. of Lond. Vol. XV, 1867, p. 433-436).—Hagenbach, Unters. über d. opt. Eigenschaften des Blattgrüns (Poggendorff's Ann. Bd. CXLI, 1870, p. 245–275).—Gerland et Rauwenhoff, Rech. sur la Chlorophyll et quelques-uns de ses dérivés (Arch. néerland., t. VI, 1871, p. 97, *ff.*).—Sorby, Various tints of autumnal foliage (Quarterly Journ. of Science, No. XXIV, Jan. 1871, p. 64–77).—Kraus, Zur

Kenntn. der Chlorophyllfarbstoffe, Spectralanalyt. Unters. Stuttg., 1872.—Sorby, On comparative vegetable Chromatology (Proceed. Royal. Soc. of Lond., Vol. XXI, 1873, p. 442–483).—Pringsheim, Ueber d. Absorptionsspectra d. Chlfarbstoffe (Monatsber. d. K. Acad. d. Wiss., Berlin, 1874, p. 628–659).—Pringsheim, Ueber natürl. Chlorophyllmodificationen, etc. (*id.*, 1875, p. 745–759).—Pringsheim, Ueber Lichtwirk. u. Chlorophyllfunction in d. Pfl. (Pringsheim's Jahrb., Bd. XII, 1880, p. 408, *ff.*).[166] [C. Timiriazeff, Comptes Rendus XCVI, 1883, pp. 375–6.]

Chlorophyll or leaf green very seldom occurs in cells in a pure state, that is, as a dissolved green pigment (Hildebrand, Weiss, Trécul; the cases are, however, still doubtful because they have not been exactly investigated), but mostly in combination with proteid substances. The latter commonly form grains, more rarely spiral bands (*Spirogyra*), or star-shaped forms (*Zygnema*). They represent the colorless fundamental substance which is covered or penetrated by the green coloring matter. On account of their prevailing granular form we commonly speak of chlorophyll grains. But the chlorophyll can easily be separated from the fundamental substance (by alcohol, benzole, etc., in which it dissolves) the colorless mass being left without having its form perceptibly changed. It consists as already mentioned in great part of albuminous substances but contains also small quantities of fats, oils, tannic acid and sugar. As is well known the chlorophyll grains are the seat of the process of assimilation. In them starch is formed, in a manner still unknown, from the elements of carbonic acid and water, as the first visible product of assimilation (in many cases fat-like substances in place of this, Sachs, Briosi). Till very recently starch has been considered the first visible product of assimilation, but according to the more recent investigations of Pringsheim the first product is a body containing oil, called Hypochlorine, which may be separated by muriatic acid and soon assumes crystal-like strata. It penetrates the whole porous proteid framework of the grain. Sachs, Hansen, Tschirch deny the

[166] See also note 144 on p. 334. A perfect list of the chemical literature concerning chlorophyll may be found in Husemann, p. 24, *ff.*

existence of hypochlorine, asserting it to be the product of the effect of the acid upon the chlorophyll. The investigations of this subject are not yet concluded. That starch may easily be made visible in chlorophyll grains we have already shown (p. 364).

In the microscopical investigations of chlorophyll grains they should be so treated as to study either the colorless ground-substance, or the coloring matter itself. We will consider both in their turn.

1. The Fundamental Substance of Chlorophyll Grains.

The fundamental substance may be investigated in the granules which are permeated with coloring matter, also—and indeed much better—in those which have been freed from the chlorophyll. For the latter purpose the portion of the plant to be investigated or a section of it should be laid in at least 90 per cent alcohol or in ether which will dissolve out the coloring matter and bleach the object. It is not recommended to boil the specimen in water as in preparing a chlorophyll solution since this coagulates the fundamental mass. The bleached grains remain behind quite unchanged in the cells. Those chlorophyll grains which contain no starch are best adapted to this investigation (for example, those of *Allium cepa* Sachs), otherwise one would naturally get the characteristic reaction of starch. Many reactions, however, are uninjured by the presence of starch.

Of the microscopical reactions the following may be referred to.[167] The bleached grains take a brick red color from acetic acid cochineal extract (Maschke): alcoholic iodine solution colors them yellow or dark brown with contraction. Put a section containing the bleached grains in a concentrated solution of copper sulphate for about half an hour, wash and transfer to a strong solution of potash and the grains become a distinct violet. Similar sections somewhat warmed in nitric acid, washed out with water, and treated with potash solution, the

[167] Mostly according to Sachs, Flora, 1863, p. 195, *ff.*

chlorophyll grains will either retain their form or be changed into a formless mass. In the former case each chlorophyll grain becomes a distinct orange yellow; in the latter case the cell is filled with an orange yellow, amorphous mass. Green leaves laid in a concentrated solution of potash for about an hour, the chlorophyll grains remain green and unchanged; wash with water and the cells will contain a homogeneous mucilage; neutralize with acetic acid and add alcoholic iodine and the cells will appear to be filled with a fine granular brownish mass. If the leaves lie for several days in the potash the chlorophyll will run together into a homogeneous layer. Bleached chlorophyll grains behave under this treatment quite the same only that they are more resistant to the action of the strong alkali than the green. Ammonia leaves the form in fresh granules quite distinct, the substance only becoming somewhat filled with spaces and vacuoles. After washing they are still almost exactly the same green; neutralize with acetic acid and add alcoholic iodine and the granules appear sharply defined, somewhat contracted with vacuoles, brown. In other cases they are less resistant toward ammonia. Phosphoric acid makes fresh chlorophyll grains yellow, but does not change the bleached grains at all. The green as well as the bleached grains are much more resistant toward sulphuric acid than is either protoplasm or the cell nucleus; the green ones become either verdigris or blue green. Cold acetic acid colors green chlorophyll grains clear yellow but leaves their form unchanged; by boiling in it they become knotty.

We infer from all these reactions that the fundamental substance of chlorophyll grains belongs to nitrogenous matter. It is a "protoplasmic form" (Sachs).

According to Pringsheim,[168] treating chlorophyll grains with dilute muriatic acid (the best is one part acid to four parts water) for a long time they become a yellow-green, gold yellow or brownish. After a longer time (several hours) their dark, reddish-brown or rust-colored periphera separates from the rest of the substance of the sharply defined mass of the chlorophyll grain. This becomes afterwards distinctly angular,

[168] Pringsheim's Jahrb., Bd. XII, p. 294.

pointed and forms a more or less extended scale or nest of indistinct crystal-like forms which throw out oblique and pointed projections. They are Hypochlorin and correspond to a mixture of oil and resin substances. They are insoluble in water and dilute acid; on the other hand they are soluble in ether, benzole and sulphuretted carbon, and vaporize at about 50°.

2. Chlorophyll Coloring Matter.

Notwithstanding the many investigations into the nature of the coloring matter of chlorophyll it is not yet satisfactorily known. By the latest investigations it appears to be established that crude chlorophyll (Rohchlorophyll, Wiesner) consists of at least a yellow and a green coloring matter (chlorophyll in the strict sense). The chemical composition of the green coloring matter is, according to Gautier, C = 73.97, H = 9.80, O = 10.33, N = 4.15, incombustible elements = 1.75. With this essentially agrees the analysis of Rogalski,[169] while those of others are very different. Essential chlorophyll is according to Gautier[170] a crystallizable body; he obtained clinorhombic crystals about ½ cm. long of soft consistency and intense green color which in the light became yellow-brown, yellowish or brownish and afterwards quite colorless.

In the study of chlorophyll coloring matter we must investigate its chemical reaction, as well as its optical (spectroscopic) behavior.

A. Behavior toward Reagents.

With the exception of a yellow or greenish decomposition product[171] which crude chlorophyll often forms in water and is soluble in that, it is insoluble in cold or boiling water as well as in dilute acids or alkalies. On the other hand it is soluble in alcohol, sulphuretted carbon, ether, benzole (Kraus), in many oils and in turpentine (Wiesner). In order to prepare an alcoholic solution of crude chlorophyll the part of the plant

[169] *Cf.* Husemann, *l. c.*, p. 251.
[170] Gautier in Comptes Rendus, LXXXIX, p. 861, *ff.*
[171] Pringsheim in Monatsber. d. K. Acad. d. Wiss. Berlin, 1875, p. 748.

to be used, preferably leaves, should be (according to Kraus)[172] put in hot water and boiled once or twice, the water poured off and boiling alcohol of 95 per cent (sp. w. 0.816) poured on. If alcohol of 83 per cent be used cold, the parts of the plant containing oils, wax, etc., will not enter into the solution (Gautier). The alcoholic crude chlorophyll extract should be fresh when used in investigations, although it is much less decomposable if the leaves have been previously boiled; apparently this manipulation removes the salts and other impurities from the leaves (Stockes, Kraus). The chlorophyll solution thus prepared is of a beautiful green color and has a dark red luster. It represents a mixture of colors which can be easily separated as Kraus[173] has indicated into a green and a yellow part.

Add to an alcoholic extract of crude chlorophyll a like quantity of benzole, vigorously shake it up and leave the mixture a short time to itself, and the alcohol and benzole will again separate. The under fluid is now a yellow-colored alcohol, the upper a green-colored benzole.[174] By this process the crude chlorophyll is separated into a yellow alcoholic part, xanthophyll (Kraus) and a green benzole part, kyanophyll (Kraus). According to Wiesner,[175] instead of the benzole one may use fatty oils (linseed oil, nut, poppy, olive oil) essential oils (turpentine, rosemary, gaultheria oil) or sulphuretted carbon.

Kraus and others therefore held that the yellow coloring matter (xanthophyll) and the blue green (kyanophyll) together represent chlorophyll; that they are both components of the same green coloring substance. According to the investigations of Pringsheim and Wiesner it appears, however, that the kyanophyll of Kraus is relatively pure chlorophyll, but that the xanthophyll of Kraus consists of yellow modifications of chlorophyll

[172] Kraus, Chlorophyllfarbstoffe, p. 23.

[173] Kraus, *l. c.*, p. 87, *ff.* The objections raised by Konrad (Flora, 1872, p. 396, *f.*) rest on insufficient experiments and have already been duly confuted by Wiesner (Flora, 1874, p. 284, *f.*).

[174] Concerning the behavior of benzole toward alcohol of various percentages, see Pringsheim in Monatsber. K. Acad. Berlin, 1874, p. 648, *f.*

[175] Wiesner in Flora, 1874, p. 282, *f.*

whose relations to crude chlorophyll are not fully established, but which as such occurs independently in it.

(*a*) *Benzole Chlorophyll* (*Kyanophyll*, Kraus). The chlorophyll procured from alcoholic solution of crude chlorophyll by the use of benzole is a beautiful green with a distinct shade of blue. It has a strong red luster, a much stronger, more carmine red luster than the crude chlorophyll solution. It is very sensitive to acids, the least trace being sufficient to change the beautiful green to a smutty yellow brown or bronze-green (Kraus). If the separation is produced by sulphuretted carbon or the above named fatty or essential oils the solution is full green and has a strong red luster. A saturated solution of chlorophyll in pure olive oil or sulphuretted carbon is a deep, almost a black-green color, and appears dark-red by reflected diffused daylight. It will keep a long time in the light if the oxygen is excluded from it; also in the dark, even if oxygen is admitted; but by the admission of oxygen to it in the light it rapidly loses its color (Wiesner).

(*b*) *The Yellow, Alcohol Part* (*Xanthophyll*, Kraus) is according to Kraus a pure gold yellow and shows no trace of fluorescence (also Gerland and Rauwenhoff, Filhol). According to Pringsheim it has a touch of green shade and is distinctly fluorescent if one examine it with a condensing lens in direct sunlight. Evaporated to dryness there remains a deep yellow-brown, sticky hydroscopic mass, which may be dissolved again in alcohol, ether, benzole and carbon sulphate, but not in water. If sulphuric or muriatic acid be added to the yellow solution it will remain yellow for a short time, then become emerald green, verdigris green and finally a beautiful indigo blue. Organic acids do not apparently alter the solution. In the sunlight it gradually bleaches out after several days. According to Kraus the gold-yellow alcohol solution is identical with the yellow coloring matter of etiolated plants.

According to the investigations of Pringsheim[176] and Wiesner it is very probable that the yellow alcohol portion of a solution of crude chlorophyll is a mixture of one or more yel-

[176] Pringsheim in monthly report of the Imperial Acad. Berlin, 1874, p. 628, *ff.*

low modifications of chlorophyll with a little chlorophyll. It first appears independently in the crude chlorophyll and is no preëxisting component of it. Pringsheim investigated three yellow modifications of chlorophyll, viz., etiolin, xanthophyll and anthoxanthin.

Etiolin is the coloring matter which is formed by etiolated growths breathing in the darkness. It is a yellow modification of chlorophyll having a red fluorescence, soluble in alcohol, ether, benzole, and carbon sulphate but insoluble in water. Its solution becomes by the addition of muriatic or sulphuric acid, first verdigris green and afterward blue.

Xanthophyll (in Pringsheim's sense) is the yellow coloring matter of autumn leaves. It behaves towards the before mentioned dissolving media quite like etiolin, but becomes emerald green not a blue, by the addition of muriatic or sulphuric acid. The yellow coloring matter of autumn leaves arises from a process of decomposition in the crude chlorophyll. If the coloring matter of the yellow xanthophyll grains be extracted by alcohol the grains remain behind in their original size. These are but gradually affected by concentrated sulphuric acid but boiling potash changes them to a greasy brown mass.[177]

Anthoxanthin is the yellow coloring matter of yellow flowers and fruit. It will be described further on.

Which of the two first yellow modifications of chlorophyll occurs in the crude chlorophyll, whether etiolin or xanthophyll, or both together, cannot be previously determined.

B. Spectroscopic Behavior of Chlorophyll.

The optical characteristics of a solution of chlorophyll have been frequently investigated, since Brewster first directed attention to the subject, and himself observed its most important phenomena, which led him to views that proved the incorrectness of some of the statements of Newton concerning the nature of light. Not to mention that he first observed the fluorescence of a solution of leaf green, he discovered its dichromism also,

[177] In many cases, however, before the appearance of the xanthophyll the chlorophyll grains pass into a beautiful green amorphous mass (Sachs, Flora, 1863, p. 202.)

that is, the quality by which a thin layer of it gives an absorption color of green and a thicker one of red. He first also observed the dispersive power of chlorophyll for red light (carefully investigated, later, by Stockes), and finally the peculiar absorption spectrum of leaf green. Of the latter, to which we here exclusively devote our attention, special and exact investigations were subsequently made by Askenasy, Kraus, Pringsheim, and others. It has been shown by these naturalists that the chlorophyll spectrum may be employed under all circumstances for the recognition of chlorophyll and its modifications, that also a spectro-analytic investigation of chlorophyll is possible, which has been much employed in the most important studies of later times.

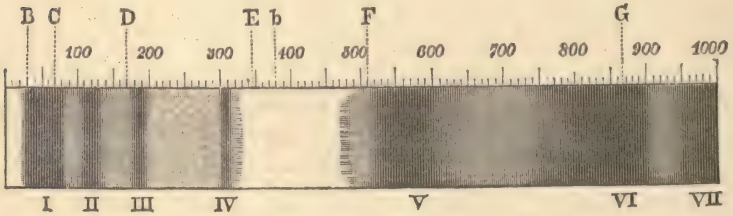

FIG. 140.

The absorption spectrum of chlorophyll shows seven dark bands, which correspond to the places of maximum absorption. The seven absorption bands are counted progressively from the red towards the blue and are designated by the Roman numerals I to VII. I to IV lie in the anterior part of the spectrum in the region of least refraction, between the Fraunhofer lines A and E (bands of the first half of the spectrum), V to VII in the posterior part or region of most refraction (bands of the second half of the spectrum). Bands I to IV are easily perceived in solutions of chlorophyll of medium concentration. Bands V to VII often give a continuous absorption, but they always appear with a sufficiently weak concentration of the solution. Fig. 140 represents an absorption spectrum with all

the bands, Fig. 141 a spectrum with a continuous absorption in place of bands V to VII (after Kraus).

The visibility, the intensity, the relative distance apart (within certain limits) of the band, are dependent on certain different factors, namely :—

The concentration of the solution, or what is equivalent the thickness of the layer of coloring matter—the optical concentration,—conditions the number and the intensity of the bands, and secondarily also their position.

The dissolving medium conditions at the same time lesser variations in the relative distance apart of the bands, as well as the rapidity or tardiness of the development of the absorption.

The kind of chlorophyll modification has no influence upon the position of the maximum and minimum of the absorption,

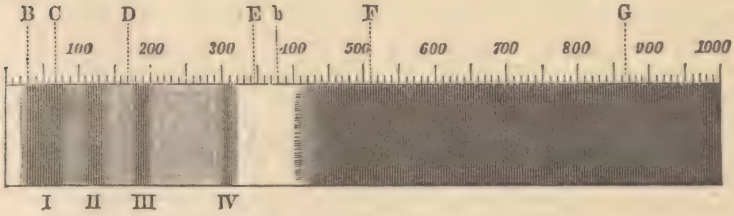

FIG. 141.

but rather upon the slower or more rapid development of the absorption within the single absorption bands.

We will consider next the spectrum of a normal alcoholic solution of chlorophyll. The anterior half of the spectrum is produced by a medium, the posterior by a weaker concentration of the solution. Fig. 140 after Kraus.

Band I, deep black, both edges sharply defined; lies between the Fraunhofer lines B and C in the red.

Band II, less black, very dark brown however abruptly shading out toward both sides, exactly in the middle between C and D in the orange.

Band III, for the most part much less dark than II, penumbrated toward both sides, in the yellow close behind the sodium line D. Between II and III a slight lessening of the light.

Band IV, very slender, weak, often scarcely visible lying before E in the green, the green behind it obscured.

Band V, broader than I, almost black in the middle, both sides shading out, lying in the light blue portion exactly behind F.

Band VI, broader than V, almost black in the middle, both sides broadly shading out, lying in the indigo, beginning in the middle between F and G and ending at G.

Band VII, corresponding to the whole of the remaining violet end of the spectrum.

FIG. 142.

That the spectrum of the alcoholic solution of the chlorophyll, here described, appertains to the coloring matter as such, that the chlorophyll extract has not been subjected to a fundamental decomposition before it is applied to the investigation, have been demonstrated by Kraus, by the fact that chlorophyll within the living plant produces similar or identical spectra.

The spectrum of a single chlorophyll grain (Fig. 142) has the appearance of a luminous spectrum through which a dark

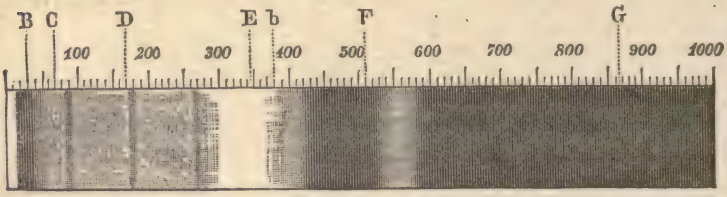

FIG. 143.

line is drawn, which is interrupted in the red and yellow. The darkening between B C corresponds to Band I. That which begins behind *b* and runs through the whole posterior part of the spectrum corresponds to the total absorption of the bands V–VII.

The spectrum of a living leaf (Fig. 143 of *Deutzia scabra*

after Kraus) is not essentially unlike the spectrum of the solution. The leaf to be investigated is put on the microscope stage and the objective shoved down till it touches it. By suitable magnification one may now recognize bands I to IV very distinctly not altered in their relative position. Band V is also sharply visible, while VI and VII are mingled in a single absorption. If a double layer of leaves is used, band VI will blend with the total absorption of the posterior half of the spectrum.

If an alcoholic solution of crude chlorophyll, of whose spectrum we have hitherto been speaking, be mixed with ben-

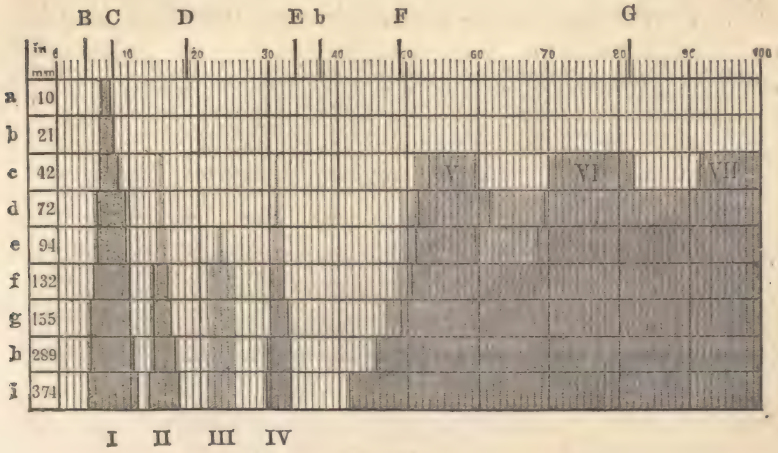

FIG. 144.

zole, the benzole portion (pure chlorophyll, kyanophyll of Kraus) will give a spectrum quite like the other, with this difference that the relative distances apart of the bands and the relative breadth of the same will have undergone some alteration. Kraus held this to be a characteristic of his kyanophyll, but according to Pringsheim, the reason for it is to be sought in the different influences of the media of solution.[178]

If by the effect of light or oxygen or of other agents (acids, etc.) a decomposition of the chlorophyll takes place, the pro-

[178] For particulars see Pringsheim (Monatsber. Berl. Acad., 1874, p. 628, *ff.*) especially also the simple and double dividing of band I of the benzole solution of chlorophyll in certain degrees of concentration.

duct of the decomposition gives a very different spectrum from that of the original coloring matter (see concerning this, Kraus, *l. c.*, p. 68, *ff.*).

By the investigations of Pringsheim it has been established that the spectrum of chlorophyll solutions of different thicknesses shows certain highly characteristic changes which may be best seen in Fig. 144, copied from Pringsheim.

The horizontal divisions, *a* to *i*, represent the spectrum of a single degree of dilution (or thickness of layer) of an alcoholic chlorophyll solution; *a* is a layer of the solution 10 mm. thick, *i* that of a like concentration 374 mm. thick. The other values are apparent from the illustration. The spectrum is divided, according to the fundamental scale of Sorby and Browning, into 100 or 1,000 parts. *B*, *C*, *D*, etc., give the position of the Fraunhofer lines; I to VII designate the absorption bands.

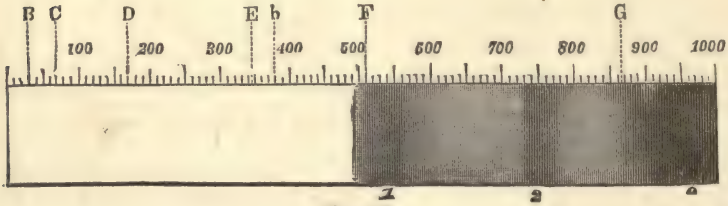

FIG. 145.

The spectrum *i* shows the bands I to IV very clearly, while V to VII run together into a single absorption. The spectra *h*, *g*, *f* are like this, only that bands I, II and IV become more narrow, and the absorption of the second half of the spectrum is drawn back more toward *F*. Spectrum *e* is distinguished by the almost total disappearance of band III and the coming out distinctly of band V. In *d*, band III has altogether disappeared, and II and IV become almost entirely clear, and V, VI and VII clearer. In *c* of the anterior bands only I is still to be clearly seen; but V, VI and VII are coming to be more distinctly perceptible. Finally, in *b* and *a*, all the bands but I appear no more. Band I is thus the most persistent, and, except that it becomes gradually narrower it remains quite un-

changed. It has, therefore, a special importance for the recognition of very dilute or much modified solutions and may be designated as the characteristic *chlorophyll band*.

The spectrum of etiolin is apparently very different from that of chlorophyll in weaker concentrations. It shows no absorption bands in the anterior half (Fig. 145, after Kraus), while beyond the line *F* are seen three absorption bands corresponding to V, VI and VII of the chlorophyll spectrum, the spaces between which are shaded. It was formerly supposed that the coloring matter of etiolized plants would not generally pro-

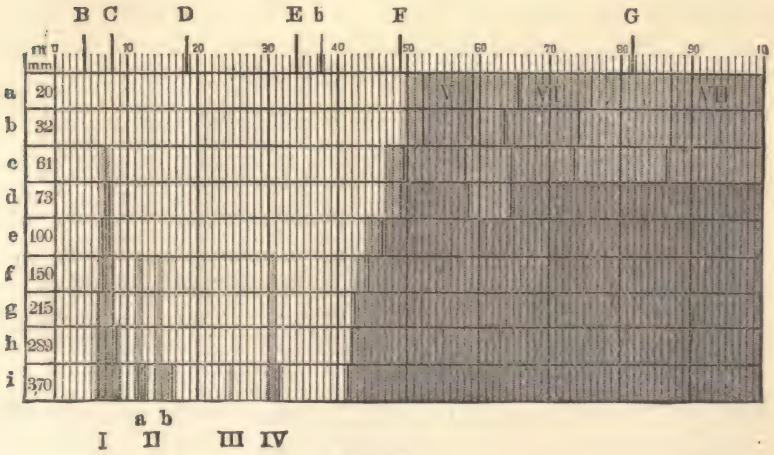

FIG. 146.

duce the bands I to IV, but Pringsheim has shown that a layer of etiolin sufficiently thick would afford a spectrum which essentially agrees with that of chlorophyll (Fig. 146 is constructed in the same manner as Fig. 144). The essential distinction lies in this, that bands I to IV are not so strongly pronounced, and appear only when using thicker layers of the solution. So also number II is divided into two bands, *a*, *b*, and the position of the bands in the blue is somewhat altered. Etiolin stands therefore optically very near to chlorophyll.

The spectrum of xanthophyll in Pringsheim's sense is much more variable. It shows only the three bands in the blue, and it

is still uncertain if even with a thickness of 370 mm. band I in the red really exists. But if the solution be much concentrated by evaporation and then tested in that thickness, there appears quite a distinct dark, but slender band I from the lithium line to near *C*, and one beginning at *E* and from *b* on becoming a very dark absorption. On the other hand bands II, III and IV have never been made to appear.

The absorption appearances in the spectrum, especially as they are produced by layers of fluid of different thicknesses, may be graphically expressed by the form of curves, the so-called absorption curves. Askenasy[179] first employed them. He drew a curve from the spectrum of a layer whose ordinates stood in relation to the intensity of the darkening, so that the maximum of the curve corresponded with the maximum of the darkening.

Without photometric apparatus this method must, however, lead to very arbitrary, or at least subjective results. It has, therefore, been but little used.

Another method introduced by Pringsheim[180] aims at the graphical representation of the maxima and minima of absorption. It brings the whole course of the absorption before the eye. See Figures 144 and 146.

The abscissa axis is divided into 100 or 1,000 parts, corresponding to the scale of the spectroscopic measuring apparatus, when the Browning scale is the standard, or directly in wave lengths in hundred thousandth parts of a mm. when the scale of Angström[181] is the standard. The ordinate axis gives the optical concentration (height of fluid layer in millimeters). There is obtained in this way a coördinate system in which the observed absorption bands may be directly registered. For the better locating of points, the position of the Fraunhofer lines, *B*, *C*, *D*, *E*, *b*, *F*, *G*, should be designated above the abscissa line,

[*Distribution of energy in the chlorophyll spectrum.*]

[C. Timiriazeff* points out the intimate relationship between the absorption of light by chlorophyll and the intensity of the

[179] Askenasy in Botan. Zeitg., 1867, Taf. V.
[180] Pringsheim in Monatsber. d. Berl. Acad., 1875, p. 795.
[181] *Cf.* Nebelung in Bot Zeitg., 1872, Taf. XI.
* In Comptes Rendus, *l. c.*, pp. 375–6.

chemical phenomena produced, the curves of absorption of light and of the decomposition of carbonic dioxide presenting an almost exact concurrence. This last function may be considered as dependent on the energy of radiation, as measured by its effect on the thermo-pilo. Langley has definitely fixed the position of maximum energy in the solar spectrum to be in the orange, exactly in that part which corresponds to the characteristic band of chlorophyll between *B* and *C*.]

[It follows, therefore, that chlorophyll may be regarded as an absorbent specially adapted for the absorption of those solar rays which have the greatest energy, and its elaboration by the vegetable economy is one of the most striking examples of the adaptation of organized beings to the conditions of their environment.]

[Under the most favorable conditions 40 per cent of the solar energy, corresponding to the rays of light absorbed by the characteristic chlorophyll bands (see pp. 153 and 413 *ff.*) is transformed into chemical work. Chlorophyll therefore constitutes an apparatus of great perfection capable of transforming into useful work 40 per cent of the solar energy absorbed.]

[Quoted from the Journal of the Royal Microscopical Society, Vol. III, No. III, p. 390. A. B. H.]

XI. THE COLORING MATTER OF FLOWERS.

Literature. Marquart, Die Farben der Blüten, Bonn, 1835. —Böhm, Physiol. Unters. ü. blaue Passiflorabeeren (Sitzungs. d. K. Acad. d. Wiss., Wien, Bd. XXIII, 1857, p. 19, *ff.*). —Wigand, Einige Sätze über die Bedeut. d. Gerbstoffe u. d. Pflanzenfarben (Botan. Zeitg., 1862, p. 121, *ff.*).—Wiesner, Einige Beobacht. über Gerb- und Farbstoffe d. Blumenbl. (*id.* p. 389, *ff.*).—Hildebrand, Anat. Unters. über d. Farben d. Blüten (Pringsheim's Jahrb., Bd. III, 1863, p. 59, *ff.*).—Weiss, Unters. über d. Entwicklungsgeschichte d. Farbstoffes in Pflzellen (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LIV, 1 Abth., 1866, p. 157, *ff.*).—Nägeli u. Schwendener, Mikrosk., p. 500, *ff.*—Kraus, Zur Kenntn. d. Chlorophyllfarbstoffe, etc., Stuttg., 1872.—Kraus, D. Entsteh. d. Farbstoffkörper in den

Beeren von *Solanum Pseudocapsicum* (Pringsheim's Jahrb., Bd. VIII, 1872, p. 131, *ff.*).—Wiesner, Unters. über d. Farbstoffe einiger für Chlorophyllfrei gehaltenen Phanerog. (*id.* p. 575, *ff.*).—Pringsheim, Ueber d. Absorptionsspectra der Chlorophyllfarbstoffe (Monatsber. d. K. Acad. d. Wiss., Berlin, 1874, p. 628, *ff.*).—Pringsheim, Ueber natürl. Chlorophyllmodificationen, etc. (*id.* 1875, p. 745, *ff.*).—Borscow, Notiz über d. Polychroïsmus einer alkohol. Cyaninlösung (Bot. Zeitg., 1875, p. 351).—Holstein, D. Schicksal d. Anthoxanthinkörner in abblüh. Blumenkr. (Bot. Zeitg., 1875, p. 25, *ff.*).—Flahault, Sur la form. des matières colorantes dans les végétaux (Bull. de la Soc. bot. de France, t. XXVI, 1879, p. 268, *ff.*).

The coloring matter of floral leaves and colored pericarps is still much less perfectly known than chlorophyll and its related substances. Like these it always appears as a part of the cell contents, never united with the membranes. Either it is dissolved in the cell sap and so represents a fluid, or it is united with variously formed granular structures, of probably protoplasmic nature. Dissolved it constitutes chiefly the blue violet and rose red colors; united with granules it is yellow, orange and green. To both cases there are, however, exceptions. These colors together often produce mixed colors.[182] Since the investigations by Marquart of the colors of flowers we designate the dissolved blue and red pigment as Anthocyan,[183] the yellow and orange colored as Anthoxanthin. Fremy and Cloëz assumed three kinds of flower coloring matter, viz. Cyanin, the blue pigment, probably identical with Marquart's Anthocyan, red coloring matter is a modification of it. Secondly, Xanthein, a yellow coloring matter soluble in water, thirdly, Xanthin, a yellow coloring matter insoluble in water, which contains a considerable quantity of fatty matter and is soluble in ether and alcohol. Identical with Xanthein is probably the Lutein of Thudichum and the Anthochlor of Prantl. According to Pringsheim, the separation of the yellow pigment is inadmissible both being according to him Anthoxanthin, a

[182] For this see Hildebrand, *l. c.*

[183] The red might also be especially set off as erythrophyll.

modification of chlorophyll similar to etiolin and xanthophyll (see p. 413, *f.*).

A. Anthocyan, Cyanin, soluble in alcohol and ether. If the dissolving medium be evaporated, the blue coloring matter may be again taken up with water. Acids color the pigment red or violet, alkalies change the red again to blue, violet or yellow green. According to Wiesner[184] anthocyan never is colored green by the use of alkalies. Where this is the case the green color is given by the presence of tannin which is colored yellow with alkali and gives the green color with the blue anthocyan (Nägeli and Schwendener, and Sachsse controvert this).

Spectroscopically anthocyan has not been very carefully tested The blue modification shows an absorption beginning at *D* and continuing to *F*, the violet a weak one at *D*, and a larger one at the blue end of the spectrum, the red an absorption band in green and blue to *F*, and an end absorption beginning at *G*.

B. Anthoxanthin (including xanthein, lutein, anthochlor). It occurs mostly in connection with proteid substances (rarely in oily substances). It is not distinguished from the colorless fundamental substance of the chlorophyll granules. With few exceptions it is insoluble in water, soluble in alcohol, ether, benzole and other media for dissolving chlorophyll. These solutions are colored blue with acids and are faintly fluorescent. According to Hanstein anthoxanthin granules are changed by the fading of the floral crown by being transformed into a yellow quite homogeneous mass.

Pringsheim[185] has shown that the spectrum of anthoxanthin itself is not essentially different from that of chlorophyll (Fig. 147). An alcoholic solution of coloring matter in thin layers shows only bands V, VI, VII, which soon run together into a continuous terminal absorption. By further increasing the contents of the coloring matter, first, band I, then II and IV, and at last band III, make their appearance. Anthoxanthin shows spectroscopically all the essential marks of chlorophyll and is to be regarded as a modification of it.

[184] Wiesner in Bot. Zeitg., 1862, p. 389.
[185] Monatsber. d. K. Acad., Berlin, 1874, p. 638, *ff.*

If the yellow coloring matter of the flowers occurs as a solution in the cells, it may be extracted by water, and colored brown yellow by the addition of potash lye.

Those peculiar coloring substances which occur in pericarps and elsewhere, mostly connected with granules, and which have been investigated by Böhm, Weiss and others, are probably to be regarded as flower pigments.

The blue granules in the fruit of the *Passiflora* are soluble, according to Böhm, in water, alcohol and ether, both cold and boiling. Potash changes their color to yellow brown. Acids and alkalies dissolve them.

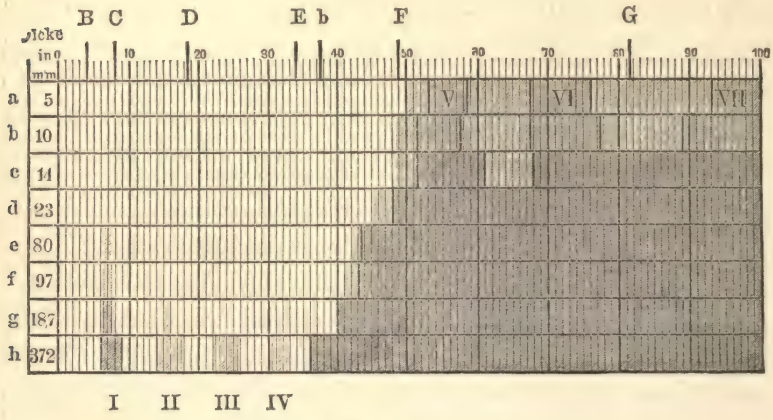

FIG. 147.

Weiss has given the reaction of numerous granules of that kind, the most important of which are the following:—

Orange colored granules. Iodine colors them green (*Cucurbita*, *Aeschinanthus*, *Canna*, *Gazania*, *Lilium*, *Hemerocallis*, *Capsicum*), or blue green (*Gaillarda*), potash does not alter the color (*Cucurbita*, *Aschinanthus*, *Gazania*) but dissolves them, sometimes when applied dilute (*Aeschianthus*), sulphuric acid colors green-yellow (*Lilium*), nitric acid first light blue then the color disappears (*Lilium*).

Yellow granules. *Adonis:* iodine does not alter them, likewise benzole, potash bleaches them somewhat. *Tydaea:* iodine colors yellow-green, potash is without effect.

Carmine red granules. Lycopersicum: iodine colors them green to yellow-green, potash leaves them unchanged. *Columnea:* iodine as well as sulphuric and muriatic acid leaves them unaltered, but coagulates them. Nitric acid makes them a vermillion red but does not coagulate them. Chlorine water is without effect.

Violet granules. Convallaria: iodine colors red, acids destroy the color. *Solanum melongena:* iodine colors gold yellow, potash blue.

Blue granules: iodine does not color them, but potash does a beautiful green (*Delphinium*).

These coloring substances arise from the transformation of chlorophyll. Plasmic forms are their fundamental substance. They give double refraction.

XII. ASPARAGIN.

Literature. Piria, Rech. sur la const. chim. de l'asparagine, etc. (Ann. de chim. et de phys., 3e sér., t. XXII, 1848, p. 160, *ff.*; abgedruckt aus Il Cimento, Jan., 1846). Pasteur, Nouvelles rech. sur les relations qui peuvent exister entre la forme cristalline, la comp. chim. etc., (*id.*, 3e sér., t. XXXI, 1851, p. 70, *ff.*)—Hartig, Entwicklungsgesch. d. Pflkeims., Leipzig, 1858, p. 128, *ff.*—Pfeffer, Unters. über d. Proteïnkörner u. d. Bedeut. d. Asparag. beim Keimen d. Samen (Pringsheim's Jahrb., Bd. VIII, 1872, p. 530, *ff.*).—Pfeffer, Ueber d. Bezieh. d. Lichtes z. Regen. v. Eiweissst. aus dem b. Keimungsprocess gebild. Asparagin (Monatsber. d. K. Acad. d. Wiss., Berlin, Dec., 1873, auch Bot. Zeitg., 1874, p. 249, *ff.*; übersetz in Ann. des sc. nat., 5e sér., t. XIX, 1874, p. 371, *ff.*)—Pfeffer in Tagebl. d. 46. Vers. dtsch. Naturf. u. Aerzte z. Wiesbaden, Sect. Bot. (Bot. Zeitg., 1874, p. 236).—Pfeffer, D. Bild. stickstoffhalt. Subst. in d. Pfl. (Jahrb. f. Landwirthsch., Bd. III, 1873, p. 437, *ff.*). Gorup-Besanez, Weitere Mitth. über d. Auftret. v. Leucin neben Asparagin, etc. (Bot. Zeitg., 1874, p. 379, *ff.*). Borodin, Ueber d. phy-

siol. Rolle u. d. Verbreit. d. Asparagins im Pflreiche (*id.*, 1878, p. 801, *ff.*).[186]

Asparagin ($C_4H_8N_2O_3$) is, it would seem, a widely distributed substance in the vegetable kingdom. It was first prepared from shoots of asparagus and was named from that plant. It is found in the milk-sap of the sprouts, stems, root-tubers, fruit and seeds. Examples of its occurrence are furnished by *Convallaria*, *Paris*, *Ornithogalum* (roots, weeds), germs, seeds, roots and the stems of numerous *Papilionaceæ* which have been grown in the dark, potatoes, althea root, seeds of *Castanea*, small shoots of the hop, sprouts of *Tilia*, *Syringa*, *Sambucus*, *Quercus* (Borodin). Especially in the germs of *Lupinus luteus* it occurs very plentifully. It is found in the living plant always in a state of solution. It was first microscopically observed by Th. Hartig and given the name "gleiss" (glister).

In respect to its physiological function there exist two opposite and incompatible theories. According to one (Pfeffer) asparagin is a transitional form between the proteid-reserve substances of the seed and the living albumen of the developing plant which aids in the transference of the nitrogen. It is produced from proteid substances and is again transformed into them. But its disappearance stands in intimate connection with the disappearance of sugar, and the presence of that is necessary also to its formation. According to the other theory (Hartig, Borodin) asparagin is the form under which generally albuminous substances pass from cell to cell. ("The 'gleiss' crystal is to a certain extent the sugar of aleuron," Hartig). Proteids, not carbo-hydrates, are decomposed in the formation of asparagin which again is changed back into albumen. This explains the fact that the plant is always poor in albuminous substances when asparagin occurs.

Asparagin crystallizes easily with one atom of water in limpid, transparent orthorhombic columns, twin forms often appearing. Fig. 148, *A*, *B*, represents two forms of asparagin crystals which have been many times observed (macroscopic according to Pasteur) *C*, *D*, *E*, *F*, some more abundant microscopic forms. It is soluble in water, acids and alkalies, but insoluble

[186] The chemical literature in Husemann, *l. c.*, p. 264.

in absolute alcohol (not in dilute), ether, fatty and essential oils.

The microscopical investigation should be conducted with absolute alcohol (or oil) (Hartig, Pfeffer Borodin). Add a drop of absolute alcohol to a section lying under the cover-glass. Then after some minutes one sees crystals shoot out, partly in and on the section, partly on both glasses and partly about the evaporating edges of the fluid. The crystals may be preserved for several days by covering the section on the slide with a superficial layer of oil.[187]

In order to test the presence of asparagin in the cells, take, according to Pfeffer,[188] a section which is thicker than a layer of cells and lay it in a watch-glass in absolute alcohol and move it quickly about. Sections with little asparagin in them should be put on a slide and the alcohol added. This may either pene-

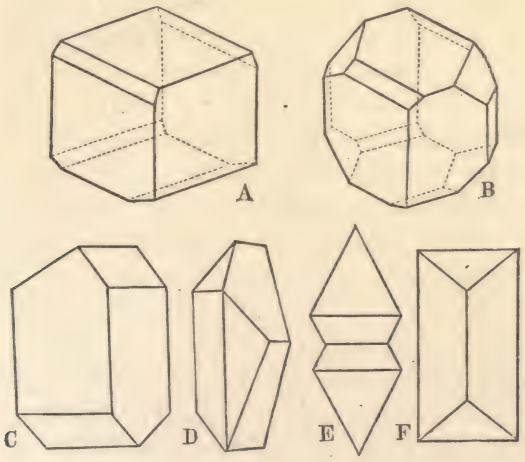

FIG. 148.

trate into the cells too quickly and so prevent the outward diosmosis of the asparagin, or it may be too dilute in the neighborhood of the section so that no asparagin can then be separated out. Alcohol should therefore be applied to the preparation

[187] Hartig, Entwicklungsgeschichte d. Pflkeims, p. 127.
[188] Pfeffer in Pringsheim's Jahrb., Bd. VIII, p. 533.

several times. According to Borodin[189] this is not a suitable thing to do. Alcohol should be applied to the section but once; lay on the cover-glass and allow it to dry. In this way a much smaller quantity of asparagin may be detected than by adding alcohol several times.

Borodin[190] gives two methods for the more certain detection of crystallized asparagin as such. Warm it to about 100° and the crystal will give up its water of crystallization and be transformed into a clear, homogeneous, strongly refractive drop, which has an outward appearance like oil and which is easily soluble in water. From this solution it may again be crystallized by means of alcohol. But if it be heated to 200° it will be so decomposed that it appears as a brown drop apparently filled with gas bubbles and no longer soluble in water.

But one may dilute the crystallized asparagin with a saturated aqueous solution of asparagin which is not colder than the object to be tested. It is best to take a cold section with a slightly warmed saturated solution and observe under the microscope the effect of a drop of it on the doubtful crystals. All other crystals soluble in water will dissolve the same in this as in pure water, but asparagin remains unchanged.

In the same manner Borodin tested tyrosin which frequently occurs in company with asparagin.

XIII. INORGANIC VEGETABLE ELEMENTS.

Literature. Raspail, Mém. de la Soc. d'hist. nat. de Paris, Sept., 1828.—Sanio in Monatsber. d. K. Acad. d. Wiss. Berlin, 1857, pp. 53, *ff.*, 253, *ff.*—Hanstein, Ueber ein noch nicht bekanntes System schlauchartiger Gefässe, etc. (*id.* 1859, p. 705, *ff.*).—v. Mohl, Ueber d. Kieselskelett lebender Pflanzen (Botan. Zeitg., 1861, No. 30, *ff.*).—Wicke, Ueber d. Vork. u. d. physiol. Verwend. d. Kieselerde (Bot. Zeitg., 1862, p. 76).—Sachs, Ergebnisse einiger neuer Unters. über d. in d. Pfl. enth. Kieselsäure, I (Flora, 1862, p. 33, *ff.*).—Sachs, do., II (*id.* 1883, p. 113, *ff.*).—Holzner, Ueber d. Kryst. in d.

[189] Borodin, *l. c.*, p. 804. [190] Borodin, *l. c.*, p. 805.

Pflzellen (Flora, 1864, p. 273, *ff.*).—Holzner, Ueber d. physiol. Bedeut. d. oxals. Kalkes (*id.* 1867, p. 497, *ff.*).—Holzner, D. Krystalldrusen in d. Bl. d. weissen Maulbeerbaumes (*id.* 1867, p. 470, *ff.*).—Rosanoff, Ueber Krystalldrusen in den Pflanzenzellen (Bot. Zeitg., 1867, p. 41, *ff.*).—Hilgers, Ueber das Auftr. der Kryst. von oxals. Kalk im Parenchym. einiger Monokot. (Pringsheim's Jahrb., Bd. VI, 1867, p. 285, *ff.*).—Dippel, D. Mikroscop., Bd. II, 1869, p. 37, *ff.*—Graf Solms-Laubach, Ueber einige geformte Vorkomann. oxals. Kalkes in leb. Zellmembranen (Bot. Zeitg., 1871, p. 509, *ff.*).—Pfitzer, Ueber d. Einlagerung v. Kalkoxalatkryst in d. pfl. Zellhaut. (Flora, 1872, p. 97, *ff.*).—Vesque, Obs. sur les crist. d'oxalate d. chaux dans les plants, etc. (Ann. des sc. nat. 5e sér., t. XIX, 1874, p. 300, *ff.*).—Sachs, Lehrb., pp. 38–66.—Penzig, Z. Verbreit. d. Cystolithen im Pflanzenreich (Bot. Centrabl., Bd. VIII, 1881 p. 393).—Also numerous statements scattered through various treatises.

The inorganic elements occurring in plants are as heterogeneous as they are wide spread. They occur in every cell membrane, in the cell contents, cell sap and protoplasm, and are the indispensable components of the body of the plant. Their presence may be detected by the incineration of a portion of the plant, after which process they remain as ashes, often indeed very minute. Commonly they are not to be recognized by means of the microscope. More seldom they appear as crystals or crystalline forms, or also as amorphic masses in the cell membrane or cell contents, and can then be discovered and investigated by means of the microscope. Only the latter therefore come within the province of our inquiry.

The elements having an inorganic basis which are visible in the cells are either silicic acid or lime (or magnesium salts) What physiological role they play is indeed a question often discussed, but as yet almost altogether unsolved. Doubtless, indeed, they have the biological function, to give to plants and to parts of plants a higher degree of solidity and a greater resistance to outside influences, especially toward the assaults of animals (silex layers in Equisetum stems, and grass blades, cell walls of diatoms, etc.). The salts of calcium are, on the con-

trary, often to be regarded as excretions or more exactly as cell excrement.

The forms to be described here are insoluble in water, but in strong mineral acids, mainly muriatic or nitric acids, either soluble or insoluble. Siliceous secretions are insoluble in mineral acids, but calcium salts are soluble in them.

A. Silex. It occurs in the cell membranes of numerous plants, in the stalk and leaves of many grasses and *Bambusæ*, in the sparkling outer layer of the *Calamites*, in the epidermis of the *Equisetæ*, in the cell walls of the *Bacillaria*. It is insoluble in acids and alkalies, and is incombustible, and in this is distinguished from every other vegetable element with an inorganic basis. We may best obtain the siliceous incrustation as a complete skeleton, by calcining the part on the platinum slip, after having first withdrawn the other inorganic salts by means of muriatic or nitric acid (for method see p. 164).

In order to obtain the siliceous frustules of diatoms beautiful and free from impurities the material should be first separated from the larger impurities by a fine metal sieve.[191] Then boil with muriatic acid with the addition of calcium chlorate, whereby the cell contents and cellulose membranes will be destroyed and the frustules will be separated. The mixture is then poured with a considerable quantity of water into a high, narrow test-tube; let it settle, pour off the fluid and replace it three or four times with pure water. There is now with the diatom frustules a small quantity of impurity in the form of yellow or colorless flakes which may sometimes be removed by boiling the material in water to which is added a piece of clean soap.

B. Calcium salts. By far the most frequently occurring inorganic element belongs to the calcium salts, and indeed the prevailing forms are calcium carbonate and calcium oxalate, very rarely calcium sulphate or phosphate (see p. 391).

Calcium oxalate, which forms most of the microscopic crystals, crystallizes in quadratic or clinorhombic, monoclinic forms. Some of the forms of crystals most frequently found are represented in Fig. 149 (*A*, *B*, quadratic, *C–F*, monoclinic forms).

[191] They are to be had of dealers in microscopic objects.

When the frequently-occurring monoclinic prisms with orthodoms are very much developed in the direction of the longer axis and very little in the direction of the transverse axis, the formation of crystalline needles takes place (raphides) which are commonly united into bundles, the needles lying parallel and near to each other (bundles of raphides). Aggregations of crystals of calcium oxalate frequently occur.

Crystals of calcium oxalate are insoluble in water, potash lye and acetic acid, soluble without the development of gas

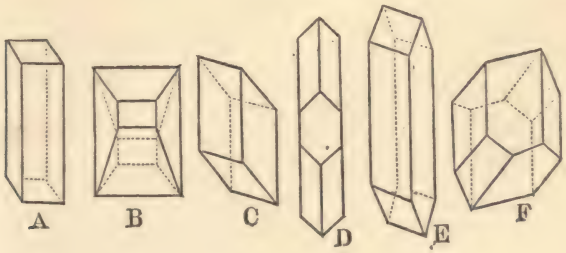

FIG. 149.

in dilute muriatic acid. If the crystals have been previously calcined they dissolve in acetic acid with the formation of gas.

Crystals of calcium carbonate are soluble in dilute acetic and muriatic acid with the development of gas. They occur now and then as the so-called cystoliths.

Crystals of calcium sulphate which have sometimes been observed are soluble in cold water.

B. PLANT SUBSTANCES OF LIMITED DISTRIBUTION.

Few of the vegetable substances of limited distribution were at first included in the domain of microscopical analysis. Whole groups, as for example, of the most important chemical as well as technical vegetable bases, are, microscopically, almost totally unknown. Others have indeed been better studied, but in respect to these also many questions still remain unsolved.

The substances described below are separated into groups which (up to the last one) correspond to their chemical behavior. They are therefore directly connected with those of the preceding section. They are: 1 Glycoside, 2 Tannic acids, 3 Alkaloids, 4 Fatty oils, 5 Essential oils, 6 Stearoptine, 7 Resin, 8 Phanerogamic coloring matter, 9 Cryptogamic coloring matter.

XIV. GLYCOSIDE.

Literature. Hartig, Ueber d. Zucker u. einem dem Salicin ähnl. Körper aus d. Cambiumsafte der Nädelhölzer (Bot. Zeitg., 1863, p. 413, *f.*).—Nägeli und Schwendener, Mikrosk., p. 494, *f.*—Franchimont, Rech. s. l'origine et la const. chim. des résines de terpènes (Arch. néerland, t. VI, 1871, p. 426, *ff.*).—Tiemann u. Harmann, Ueber d. Coniferin, etc. (Ber. Deutsch. Chem. Gesellsch., Bd. VII, 1874, p. 608, *ff.*).—Tangl, Vorläuf. Mitth. über d. Verbreitung d. Coniferins (Flora, 1874, p. 239, *ff.*).—Müller, Ueber Coniferin (*id.* p. 399.)—Borscow, Beiträge z. Histochemie der Pfl. (Bot. Zeitg., 1874, p.17, *ff.*).—Pfeffer, Hesperidin, e. Bestandth. einiger Hesperideen (*id.* p. 529, *ff.*).—v. Höhnel, Ueber d. Kork u. verkorkte Gewebe überhaupt (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXVII, 1 Abth., 1877, p. 700, *ff.*).—v. Höhnel, Histochem. Unters. über d. Xylophilin u. d. Coniferin (*id.* p. 699, *ff.*).—Schwartz, Chem-botan. Studien über d. in den Flechten vorkomm. Flechtensäuren (Cohn's Beiträg. z. Biologie d. Pfl., Bd. III, 1880, p. 249, *ff.*).—Singer, Beitr. z. näheren Kenntniss d. Holzsubstanz u. verholzt. Gewebe (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LXXXV, 1 Abth., 1882, p. 347, *ff.*).

Under this designation is included that series of vegetable substances which are produced by the action of dilute alkalies or acids on cane and grape sugar and their relatives (not including others produced therefrom by dividing the sugar molecule). Most of these are in a pure state crystallized and soluble in water; many also in alcohol; others are insoluble in the latter

and may be separated by means of this. Of the numerous bodies belonging to this group the microscopical investigation of the following has been attempted: Coniferin (abietin), vanillin, salicin, hesperidin, frangulin, syringin and chrysophonic acid (of the latter it is still doubtful if it belongs to this group). Most of the following statements require still further verification.

1. Coniferin (Abietin) $C_{16}H_{22}O_8$.

It crystallizes in white or yellow needles, soluble in water, not easily soluble in alcohol and ether. With concentrated sulphuric acid it gives a violet-blue color which by the subsequent addition of water becomes blue. According to Franchimont, coniferin can be detected in the cells by means of sulphuric acid, giving them a purple-violet coloring. It becomes green with muriated carbolic acid (more particularly as to this substance as well as its occurrence in lignified membrane, see pp. 340–1, *ff.*).

Hartig's abietin is probably identical with this. It is with difficulty soluble in water and ether, easily soluble in dilute alcohol. It occurs in the cambian sap of many *Coniferæ* and can be detected by treating a section of this wood with concentrated sulphuric acid. It shows itself in a characteristic violet blue color in the whole region of the bast ring.[192]

2. Vanillin. $C_8H_8O_3$

White crystal needles soluble in much water, alcohol and ether, becoming yellow with concentrated sulphuric acid (with iron chloride, dark violet). According to Singer it constantly occurs in lignified cell membranes and affords the well-known reaction of wood substance (see p. 331 *ff.*).

3. Salicin. $C_{13}H_{18}O_7$.

Likewise crystallizes (orthorhombic); insoluble in ether, sol-

[192] Hartig in Bot. Zeitg., 1863, p. 414.

uble in water and alcohol, still more easily in potash water as well as in acetic acid. It occurs in the bark of many species of *Salix* and *Populus*. With concentrated sulphuric acid it is colored a beautiful red (with the addition of water the color is removed with the formation of a red powdery precipitate : Rutilin).

4. Hesperidin. $C_{25}H_{30}O_{13}$ (?).

A glycoside in the ripe and unripe fruit and other parts of the orange tree. It is insoluble in water and in dilute acids, easily soluble in potash. It occurs in the cells in a state of solution, and after lying in alcohol it is precipitated in the cells in the form of spherical crystals (also in glycerine), which dissolve in alkalies with a yellow or reddish color.

5. Frangulin (*Rhamnoxanthin*) $C_{20}H_{20}O_{10}$ (?).

This occurs in the peripheral portion of the pith of *Rhamnus frangula*, also in the woody parenchyma of the pith, in the thin walled phloëm elements and in the bast parenchyma. Frangulin is a crystallizable body easily soluble in alcohol. In the cells are very small starch grains which bear the frangulin, and which give, with iodine, the characteristic blue color. The grains color with ammonia or potash lye a beautiful blood red.

6. Syringin $C_{19}H_{28}O_{10}$.

Crystallizes in fine white needles which have a kind of silky sparkle. Easily dissolves in concentrated sulphuric acid whereby the solution first becomes yellow green, then blue, and at last violet red. This quality is very useful in microscopically testing syringin. To the transverse or longitudinal section of the twig of *Syringa vulgaris* (in which syringin occurs) on the slide, add relatively concentrated sulphuric acid (one drop H_2SO_4 and two drops H_2O).

As soon as the section is penetrated by this solution immediately the entire cell wall of the wood, bast and medullary ray

cells are colored a yellow green; after a few minutes this color passes over into blue and finally into violet red. The cell membranes of the rest of the tissue as well as the cell contents remain quite colorless. By the use of still more dilute acid, however, the reaction occurs, more gradually (after two or three hours). Further, the age of the cell membranes is not a matter of indifference; the younger take the color much more rapidly than the older ones. The reaction produced by the acid soon becomes indistinct, in that by a subsequent apparent diffusion it soon also colors the cell contents red violet. Syringin occurs in the cell walls of thick walled phloëm, xylem and xylem-medullary-ray cells (Borscow).

7. Chrysophanic Acid (Rheïn) $C_{15}H_{10}O_4$.

It crystallizes in beautiful orange yellow needles which sparkle like gold. It is scarcely soluble in cold water, but is soluble, on the contrary, in ether and benzole. Alkalies produce with it in solution a beautiful purple red color. It is found in the thalus of several lichens as well as in roots of Rhabarber, *Rumex obtusifolius*, *R. patientia* and in *Cassia bijuga* (rind). In the cells it is connected with small plasma grains which take on a dark purple red color with ammonia. In the young lateral roots of *Rumex obtusifolius* it occurs in the parenchyma of the outer rind, in the thin-walled phloëm elements as well as in the thin-walled parenchyma cells. In the older lateral roots the rind parenchyma is quite free from chrysophanic acid, but it occurs, on the contrary, in the parenchyma of the pith (Borscow).

XV. TANNIC ACID (TANNIN).

Literature. Sachs, Ueber einige neue mikrosk.-chem. Reactionsmeth., II Ueber mikrosk. Nachweisung d. Gerbst. in d. Zellen (Sitzungsber. d. K. Acad. d. Wiss. Wein., Bd. XXXVI, 1859, p. 23 *ff.*).—Sanio, Einige Bemerk. üeber d. Bau d. Holzes; V, Ueber Gerbstoff (Bot. Zeitg., p. 213 f.).—Wigand, Einige Sätze über d. physiol. Bedeut. d. Gerbstoffes, etc. (*id.*, 1862, p. 121 *ff*).—Wiesner, Einige Beobacht. über Gerb-. und

Farbst. d. Blumenbl. (*id.*, p. 389 *ff.*).—Sanio, Einige Bemerk. über den Gerbstoff u. seine Verbreitung b. d. Holzpfl. (*id.*, 1863, p. 17, *ff.*).—Hartig, Ueber d. Gerbmehl (*id.*, 1865, Nr. 7.).—Nägeli u. Schwendener, D. Mikrosk., p. 490 *ff.*—Hanstein, Ueber d. Org. d. Harz und Schleimabsonderung in d. Laubkn. (Bot. Zeitg., 1868, p. 721 *ff.*).—Dippel, Das Mikrosk., Bd. II, p. 20.

Under this name we include a series of chemical combinations which, like the carbo-hydrates, consist of carbon, oxygen, and hydrogen, but which are much richer than they in carbon and oxygen. They are still but little known as to their chemical constitution.

Tannic acid occurs especially in woody growths and perennial herbs, rarely in annual plants, more frequently in dicotyledons than in monocotyledons. Whole families of plants lack tannin as *Solaneæ*, *Oleaceæ*. On the contrary, the representatives of the *Cupuliferæ*, *Ericaceæ*, *Leguminoseæ* and *Rosaceæ* are especially rich in them. Tannic acid is found chiefly in the bark, in young wood, in the thin walled vascular bundles, rarely in the pith.

Originally tannic acids always occur dissolved in the cell sap, but may, in later stages, penetrate and permeate the cell wall. They appear also to pass from cell to cell. Another kind of tannic acids (bark of the oak, etc.) is, however not capable of diosmosis. Their further progress comes about only when they shall have been transformed into other products. According to Wigand there exists a certain connection between tannin and starch. It often occurs in great abundance in the pulp of fruit, but it subsequently disappears in the mass as the sugar increases so that a transformation of tannin into sugar seems to take place. In other cases the tannins are to be regarded[193] as chromogens out of which the blue and red colors of flowers are subsequently developed.

The regular appearance of tannic acids in certain pathological cell growths (gall apples) is well known.

All tannic acids are soluble in water and in alcohol and have an astringent taste.

[193] Wigand in Botan. Zeitg., 1862, *l. c.*

There are some very characteristic microscopical reactions for the tannic acids by which they may be easily and safely recognized.

With iron salts, ferric acetate, ferric vitriol, ferric chloride, and others, tannin gives a dark blue or green color or even a precipitate with these colors. Formerly it was thought that the blue and green shades were produced by different kinds of tannin, and they were therefore divided into iron green, and iron-blue (both are sometimes found united in the same cell). According to the later investigations of the chemist, however, there are no sufficient grounds for this division. It is best to employ in the reactions a not too concentrated solution of the iron salt. Lay the section immediately into it and examine it at once. Or one may bring the reagent to the section as it lies in glycerine. One should work quickly in order to prevent the tannin solution from passing by diosmosis, out of the cells into the water used in the preparation, and so make the reaction indistinct. In the freshly prepared specimen the cell membrane commonly remains colorless, but if it have already remained a long time in water the cell membrane will be colored on account of the tannin having penetrated it. In many cells the tannin mass has an oily appearance, and separates by the addition of water into small globules (bark of oak, poplar). The blue color produced by the iron chloride assumes here at last a brown shade, from which we infer that along with the iron-bluing tannin there occurs some other substance, or another less sensitive tannin in the oily masses just mentioned.[194]

A second reagent for tannic acid is according to Sachs[195] potassium hydroxide. It produces an oxidation product of tannin which is a brick red, yellow red or red brown fluid. This, like the precipitate of iron salts, is clearly visible even in very thin sections and with the presence of very little tannin. Naturally here and there sections used for investigations are more than a single layer of cells thick. Since the red coloring with potash rests upon an oxidation process one must commonly wait for several minutes for the reaction to take place. Lay the slide

[194] Nägeli u. Schwendener, *l. c.*, p. 492.
[195] Sachs, *l. c.*, p. 27, *ff.*

with the section on white paper and cover the latter with a drop of strong potash solution. In order to convey air necessary to the oxidation of the tannin cells a few drops of water should be added. However, without this, the coloring takes place though much more gradually.

In potassium iodide of iodine, tannin takes on a yellow or yellow brown color, with dilute chlor-iodide of zinc solution there appears a reddish, rose red or red brown, or even a violet precipitate.[196] These colors are distinctly recognizable in the presence of a very little tannin.

A solution of potassium bichromate colors tannin dark red or red-brown. This red-brown combination does not dissolve in an excess of the reagent. Sanio[197] laid fragments of the branches which had been previously dried for a few hours, in the dilute reagent and prepared the sections from these when they had become thoroughly penetrated with the fluid. But the section can be impregnated with the salt on the slide.

Hanstein's aniline solution colors tannin roe-brown in bright or strong shades (Hanstein).

Gallic acid, according to Sachs, will give with baryta solution a gray blue precipitate; with chlor-iodide of zinc it gives a rose red precipitate.

XVI. ALKALOIDS.

Literature. Borscow, Beitrage. z. Histochemie der Pfl. (Bot. Zeitg., 1874, p. 17 *ff.*).

The attempt is now made for the first time to subject to a microscopical test a substance, belonging to a group of vegetable bases, which the chemist has very carefully studied, viz.:

Veratrum $C_{32} H_{52} N_2 O_8$.

The alkaloid of *Veratrum album* and *V. sabadilla* is a white crystalline powder which is insoluble in water but soluble in alcohol, ether, chloroform, benzole and glycerine (with difficulty). Concentrated sulphuric acid dissolves it with a yellow

[196] Sanio in Bot. Zeitg., 1863, p. 214.
[197] Sanio, idem, p. 17.

color, this soon becomes orange, then blood red, finally carmine red or smutty violet. Borscow[198] makes use of fine transverse and longitudinal sections to which he adds sulphuric acid, which contains a double volume of water in order not to destroy the delicate tissue. Then the characteristic color and change of color appear. The under-ground parts of the *Veratrum* were tested in this manner, and it was found that in the roots and in the continuation of the axis beneath the bulb, is the principal seat of the alkaloid in the elements of the epidermis and the protecting layer, and that in the scales of the bulb only the epidermal layer contained a little veratrum. The veratrum appears to occur principally in the inside of the cell wall.

XVII. FATS.

Literature. Karsten, Ueber d. Ensteh. d. Harzes, Wachses, etc., durch d. assimil. Thätigkeit d. Pflzellen (Bot. Zeit., 1857, p. 313, *ff.*).—Kützing, Grundz. d. philos. Bot., Bd. I, a. v. O. —Sachs, Ueber d. Auftreten d. Stärke bei d. Keimung ölhaltiger Samen (Bot. Zeitg., 1859, p. 177, *ff.*).—Sachs, Ueber d. Stoffe, welche d. Material z. Aufbau d. Zellhäute liefern (Pringsheim's Jahrb., Bd. III, 1863, p. 183, *ff.*).—Wigand, Ueber d. Desorganisation d. Pflanzelle, etc. (Pringsheim's Jahrb., Bd. III, 1863, p. 155, *ff.*).—Müller, Unters. über d. Vertheil. d. Harze, etc. in Pflanzenkörper (*id.*, Bd. V, 1866, p. 387, *ff.*).—Uloth, Wachsbildung im Pflanzenreich (Flora, 1867, p. 385, *ff.*)— De Bary, Ueber d. Wachsuberzüge d. Epidermis (Bot. Zeitg., 1871, p. 128, *ff.*).—Pfeffer, Unters. über d. Proteinkörner, etc. (Pringsheim's Jahrb., Bd. VIII, 1872, p. 419, *ff.*).—Pfeffer, D. Oelkörper d. Lebermoose (Flora, 1874, p. 2, *ff.*).—Wiesner, Ueber d. Krystallin. Beschaffenheit der geformten Wachsuberzuge pflanzl. Oberhäute (Bot., Zeitg., 1876, p. 225, *ff.*).

The fats are diffused vegetable substances of a peculiar appearance. They are either fluid (fatty oils) or they are solid bodies (wax). The fatty oils will in most cases represent reserve substances. They occur therefore in a great number of

[198] Borscow, *l. c.*, p. 38, *ff.*

resting seeds (Sachs, Pfeffer, see p. 382). The waxes seem never to be reserve material, but they appear rather in a number of cases to have certain biological functions. Where they cover the surface of growths they prevent the penetration of dampness since they are impervious to water. That the cuticle is often covered and permeated with wax has been shown by De Bary.[199]

1. Fatty Oils.

Fatty oils occur in the cells in the form of globular drops which are easily recognized on account of their refractive qualities. They are insoluble in water, somewhat soluble in alcohol, easily soluble in ether, benzole, sulphurated carbon, and acetic acid. All alkalies destroy them by saponification (formation of alkali salts).

Osmic acid and alcanna tincture are the principal microscopical tests for fatty oils.

Osmic acid colors the drops of fatty oils a deep brown or black brown, alcanna tincture a beautiful red (for method see pp. 310 and 385). In order to distinguish the drops colored red by the alcanna tincture from resin drops or drops of essential oil (which are colored by it in the same way), add a little absolute alcohol, in which if the drops are not dissolved, they are shown to be fatty oil.

Concerning the somewhat variable behavior of the oil drops of the *Hepaticæ* toward dissolving media see Pfeffer in Flora, 1874, p. 2, *ff.*

2. Wax.

Wax occurs as a solid whitish or yellowish, sometimes crystalline crust on the surface of stems and leaves. (*Acer*, *Ceroxylon andicola*, *Myrica cerifera*, *Klopstockia*, *Liriodendron tulipifera*, *Eucalyptus*, *Acacia cultriformis*, etc.) and on the fruit of many plants, as the so-called "rime." It is insoluble in water, a little soluble in cold alcohol, soluble in boiling alcohol, ether, chloroform and essential oils. Alkalies and acids

[199] De Bary in Bot. Zeitg., 1871, *l. c.*

change it scarcely at all. It often appears to arise from a metamorphosis of cellulose. Iodine and sulphuric acid do not color it, or if at all, slightly yellowish.

There are, as yet, no microscopical methods of reaction on wax.

XVIII. ESSENTIAL OILS.

Literature. Elsewhere given; partly with the fats, partly with the resins.

Colorless, yellow, red, brown or otherwise colored, strongly refractive fluids within the cells, altogether filling them or forming single drops in the cell sap, perhaps standing in certain relations to the resins. They are but slightly soluble in water but easily soluble in alcohol, especially in absolute alcohol as well as in ether. With sulphuric acid they commonly take on a brown coloring.

They may be microscopically tested by alcanna tincture or osmic acid, with which they give the same reactions as the fatty oils but are distinguished from them by their solubility in alcohol.

XIX. STEAROPTENE (CAMPHOR).

Literature. Borscow, Beitrage zur Histochemie der Pflanzen (Botan. Zeitg., 1874, p. 17 *ff.*).

Of the group of the stearoptenes, there appears to have been microscopically investigated but one body hitherto, viz.:

Asaron $C_{20}H_{13}O_5$ (?).

It is a crystallized, volatile combination which occurs, principally in the rhizomes, in *Asarum Europeum*. It is insoluble in water, easily soluble in alcohol, ether, fatty and essential oils. Concentrated sulphuric acid and fuming nitric acid color it orange red or orange yellow. Only the first named acid can be employed in the microscopical investigation. It is thereby shown that asaron occurs in the rhizomes, especially in the peripheral layer of the fundamental parenchyma, in cells which outwardly demonstrate their asaron contents by being filled with a greenish

strongly refractive substance (asaron dissolved in an essential oil not very well known), To a section lying in water add a drop of concentrated sulphuric acid and it will gradually color the whole of the drops of oil first yellowish, then pure yellow, and finally orange. If there are several oil drops in the cell they will, after treatment with the acid, run together to form one or two drops of larger dimensions.

XX. RESIN, BALSAM, TURPENTINE.

Literature. Karsten, Ueber d. Entstehung d. Harzes, Wachses, etc. (Botan. Zeitg., 1857, p. 313 *ff.*).—Wigand, Ueber d. Desorganisation d. Pflzelle, insbes. über d. physiol. Bedeut. v. Gummi u. Harz (Pringsheim's Jahrb., Bd. III, 1863, p. 115 *ff.*).—Dippel, Z. Histologie d. Coniferen II (Botan. Zeitg., 1863, p. 253 *ff.*).—Schacht, Ueber ein neues Secretionsorgan im Wurzelst. v. *Nephrodium Filix mas* (Pringsheim's Jahrb., Bd. III, 1863, p. 352 *ff.*).—Wiesner, Ueber d. Entsteh. d. Harzes im Innern der Pflzellen (Sitzungsber. d. K. Acad. d. Wiss. Wien, Bd. LI, 1865; see Chem. Centralbl., 1865, p. 756 *ff.*).—Muller, Unters. über d. Vertheilung d. Harze, etc., im Pflanzenkörper (Pringsheim's Jahrb., Bd. V, 1866, p. 387 *ff.*) Hanstein, Ueber d. Organe d. Harz- u. Schleimabsonderung in den Laubknospen (Botan. Zeitg., 1868, p. 697, *ff.*).—Franchimont, Recherches sur l'origine et la constitution chim. des résines de terpènes (Arch. Néerland, t. VI, 1871, p. 426, *ff.* see also Flora 1871, p. 225 *ff.*).—Vogl, Ueber den Bau des Holzes von *Ferreira spectabilis* u. d. Bildungsw. des sogen. Angelinpedraharzes (Pringsheim's Jahrb., Bd. IX, 1873, p. 277 *ff.*).

Principally in the group of coniferous plants, but also in very many other growths even in some cryptogams themselves (Ferns, Schacht), are found special conduits (resin ducts), which are produced by the separation or absorption of the cells. In these the substance which we call resin or balsam collects; sometimes it is poured out as a secretion through ruptures or cracks. In some cases it will be conveyed to the outer world

through peculiar organs of secretion (conduits of leaf buds, resin in *Hedera*).

Concerning its production, two views prevail. According to one, which is held by many chemists, resin is formed in the plants from essential oils. According to the view of Wiesner and others, it is produced from cellulose and starch, which are transformed as a connecting link first into tannin then into resin. According to Franchimont it is formed from the glycosides. These undergo a transformation into tannin and oxalic acid; the tannic acid, which often appears under the form of globules,[200] yields, under the influence of albuminoid matter, a substance (retinogen) which, on its part, is capable of producing, by the influence of air, resin and oil of turpentine.

Many resins of the *Coniferæ* may be designated by the formula $C_{20}H_{30}O_3$ and may be considered the oxydation products of terpene (for example of the oil of turpentine, $C_{10}H_{16}$). That which we commonly name resin is a solution of resin in its narrow sense in the terpenes (a mixture of carbohydrates, as oil of turpentine). To the resins belong also the balsams. We designate with this latter name commonly those resins, which, on account of their larger turpentine contents (about 24 per cent) are more fluid, as, for example, our balsam of fir.

Resin appears in the plant either as a fluid or as a more or less solid granule (resin-meal, Wiesner, as in the pith cells and wood parenchyma, for example, in *Acer*, *Ulmus*, *Fagas*, *Quercus*, *Protea*). Not infrequently also there occurs a mixture of gum with resin which we distinguish as gum-resin or resin-gum (see p. 373).

Resins are seldom colorless, mostly yellow or brownish and burn with a sooty flame. All are insoluble in water; many are soluble in alcohol (giving a milky precipitate by the addition of water); others are insoluble in it. Ether dissolves most resins easily, likewise sulphuretted carbon, oil of turpentine, benzole, chloroform and essential oils. (The resins of all the indigenous *Coniferæ* are soluble in all the dissolving media named.) Alkalies and mineral acids also dissolve the resins more or less easily.

[200] Qui paraissent devoir leur origine aux noyaux cellulaires (! !).

At the present time the following methods are proposed for the microscopical testing of resins.

According to Unverdorben many resins form a green combination with copper salts. On this characteristic, Franchimont[201] founds the following experiment which is applicable to many but not to all resins.

Put the whole of the part of the plant containing resin for several days in an aqueous saturated solution of copper acetate.[202] Wash it out in water and prepare the section from it. The resin canals appear then of a beautiful emerald green color.

With alcanna tincture resin colors a beautiful cinnabar red. The process to be followed here has already been described on page 310. One can use the tincture itself applying a drop to the preparation which is being tested. The specimen must be put into glycerine before the alcohol is fully evaporated, because otherwise the resinous alcanna tincture will collect itself in the form of minute drops on the walls of the cells and the results be impaired.

Hanstein's aniline solution colors many resins a beautiful blue, and it is indeed a pure blue without violet or green. It colors many balsams a verdigris green, also pure or smutty olive-green.[203]

With concentrated sulphuric acid the contents of many resin ducts become red or brown-red. The resin of many species of *Araucaria* take a similar color with sulphuric acid or potassium bichromate.

Resin meal. It is represented by globular or flattened granules of 0.0018 to 0.018 mm. in diameter, which are homogeneous within or contain a nucleus of a different refractive power; one seldom remarks a lamination in them. They are not altered by distilled water, but by boiling some remain unchanged and others are melted. Dilute alcoholic iodine does not alter them, only some take a faint, blue color. Boiling alcohol or ether dissolves but a few grains altogether, most of

[201] Franchimont, *l. c.*, t. VI, p. 427, *ff.*

[202] According to Franchimont one may also recognize other substances by means of the copper acetate. Tannin becomes brown therewith and glycose separates out metallic copper.

[203] Hanstein, *l. c.*, p. 747.

them only becoming clearer and stratified. Potassium lye dissolves them with saponification and brown coloring, as also does ammonia, though the latter works less intensively. In cuprammonia they are unchanged, likewise they are very resistant towards muriatic and nitric acid. With sulphuric acid they often become brown or dark red; with muriatic acid, red; but in nitric acid they bleach out. In dilute chromic acid many granules are disintegrated and most of them become distinctly laminated. Those which have lain for a long time in chromic acid are colorless and show the reaction of cellulose with iodine and sulphuric acid, or with cuprammonia. Ferric chloride gives with an olive green or deep blue coloring the tannin reaction.

Gummy resin. The study of this substance is best prosecuted by the help of Hanstein's aniline mixture. The preparation treated with it and carefully worked shows the resin beautifully blue, while the gums, the amyloids and other muculent carbohydrates, which appear in the blue resin in the form of large or small drops, either remain uncolored or take on a pale rose-red or strong reddish color, the specimen giving a beautiful image when thus prepared.[204]

1. Betulo-resin acid.

Literature. Müller, Einige Bemerk. über d. harzartigen Ausscheidungen an den Birken (Botan. Zeitg., 1845, p. 793).—Kosmann in Journ. d. Pharm. (2), Bd. XXVI, p. 107.—Mikosch, Ueber d. Organe der Ausscheidung d. Betuloretinsäure an der Birke (Oesterr. Bot. Zeitschr., 1876, p. 213, *ff.*).

This resin acid ($C_{36}H_{66}O_5$) is insoluble in water, soluble in alcohol, ether, alkalies, carbonic acid alkalies and concentrated sulphuric acid (in the latter with a red color). It is secreted by the hair glands on the leaves of *Betula alba*, and indeed the secretion takes place by the raising up of the cuticle. The contents of the secreting cells is first a homogeneous green (resting on the chlorophyll), but afterwards this color is displaced by a deep red-brown. The secretion is a pale yellow, syrupy mass,

[204] See Hanstein, *l. c.*, Taf. XI, Fig. 23.

in which the betulo-resin acid is found dissolved, in some manner as yet unknown. It separates as a solid from it. The contents of the younger glands are colored yellow with concentrated potassium lye, afterwards brick red (Mikosch).

2. Milk-saps.

Literature. Weiss und Wiesner, Beitr. z. Kenntnisse d. chem. u. physik. Natur des Milchs. d. Pfl. (Bot. Zeitg., 1862, p. 125, *ff.*).—Hanstein, D. Milchsaftgef. u. verwandten Organe d. Rinde, Berlin, 1863.—Dippel, Beitr. z. Histologie d. Pfl. 1. D. milchsaftführenden Zellen der Hollunderarten (Verh. d. naturhist. Ver. d. pr. Rheinl. u. Westf., 1865, Jahrg. XXII.—Dippel, Entstehung. d. Milchsaftgef. u. deren Stellung im Gefässbündelsystem. Rotterd., 1865.—Vogl, Ueber Milchsaftgef. d. Klette (Botan. Zeitg., 1866, p. 193, *ff.*).

The milk-saps represent a mixture of different kinds of materials. It is therefore difficult to arrange these plant substances in respect to their chemical qualities. But since it appears that resin always occurs in them, we have concluded to say a few words concerning them in this section.

Weiss and Wiesner[205] give as elements of the milksaps of *Euphorbia platyphylla:* resin, caoutchouc, essential oils, albuminous matter, starch, sugar, fat, extractive matter, tartaric acid, mineral elements and a coloring matter.

Milk-sap coagulates with a red coloring on coming to the air. With iodine solution it runs into yellow colored balls or brown masses. Ammonia turns it greenish (the development of the coloring matter), sulphuric acid a beautiful yellow with coagulation.—"Put a drop of sulphuric, nitric or muriatic acid on a slide, and then let a small drop of the milk-sap fall upon it and it will always spread out into a disk-shaped form and these little disks are either beautiful yellow (with sulphuric acid) or almost colorless, only very little yellow (with nitric acid), or again almost colorless with a dull shade of yellow-red (with muriatic acid). If one puts a drop of the milk-sap on a

[205] Weiss and Wiesner in Bot. Zeitg., 1862, p. 126.

solution of iodine and observes the disk thus produced with reflected light, it appears to the unaided eye a beautiful ultramarine blue. Examination with the microscope shows that this blue does not rest upon the amylum in the milk-sap, since the disk by transmitted light is no longer blue but yellow" (Weiss and Wiesner, *l. c.*).

XXI. THE COLORING MATTER OF FLOWERING PLANTS.

Literature. Decaisne, Rech. anat. et phys. sur la Garance et le dévéloppem. de la matière colorante, Bruxelles, 1837.—Nägeli u. Schwendener Mikroskop., p. 503, *ff.*—Weiss, Allgem. Bot., Bd. I, p. 137.

The coloring substances to be described here constitute the color of certain roots, woods, seed scales, seeds, etc. They have been on the whole but little investigated. It seems, however, that at first they appear on the inside of the cell dissolved in the cellulose and afterwards passing into the membrane, became intercalated and permanently remain. We will here describe some of those which have been better investigated, on the authority of Nägeli and Schwendener.

1. *Coloring matter of the Rubia tinctorum.* Sections of the fresh root appear yellow and not red; most of the bark cells contain a yellow fluid which in the air forms red flakes. Young roots possess altogether colorless membranes, the pigment being found alone in the cell contents. Potash colors it purple red, acids orange color, ferric chloride orange and at last brownish red, alcohol extracts the yellow coloring matter not the altered red.

2. *Coloring matter of Colored Woods.* Well-known examples of colored woods are the Brazil wood, sandal wood, blue wood, Pernambuco wood, etc. The coloring matter permeates the walls, and occurs also in the form of small granules, in the medullary rays and single wood cells.

In microscopic sections the coloring matters appear yellow to red orange. They are soluble in alkalies (with red, blue or violet color, acids restore the original color). They are like-

wise dissolved in water, alcohol and ether as well as in glycerine. The aqueous solutions are mostly red or bluish, the alcoholic yellow or orange. Acids dissolve them with yellow, carmine or blood red coloring. The aqueous extract of the red or blue woods are colored with ferric chloride; first, yellow, then blue (tannin reaction).

3. *Coloring matter of the Barberry-root.* This pigment is likewise at first dissolved in the cell-sap and passes from there into the membrane which it colors yellow. Dilute acids separate out small yellow drops (albuminous combinations). Potash causes a yellow precipitate and gradually extracts from it an orange yellow colored pigment. In a cold aqueous extract of the coloring matter muriatic acid produces radiating groups of crystal needles (berberidin).

4. *Red coloring matter of Abrus precatorius.* The scarlet red coloring is produced by a red pigment which is intercalated in the thick walls of the palisade-like cells which form the upper surface of the testa. Alkalies color it blue, acids a high red. It behaves also like anthocyan (see p. 423).

XXII. THE COLORING MATTER OF THE CRYPTOGAMIC PLANTS.

1. The Coloring Matter of Algæ.

Literature. Kützing, Phycologia generalis., 1843, p. 21.—Kützing, Grundz. d. philos. Bot. Bd. I, p. 166.—Cohn, Beitr. z. Physiol. d. Phycochromaceen u. Florideen (Schultze's Arch. f. mikrosk. Anat., Bd. III, 1867, p. 1, *ff.*).—Nägeli u. Schwendener, Mikrosk., p. 497, *ff.*—Askenasy, Beitr. z. Kenntn. d. Chlorophylls u. einiger dass. begleit. Farbstoffe (Bot. Zeitg., 1867, p. 225, *ff.*).—Rosanoff, Observ. sur les fonctions et les propriétés des pigments de diverses algues (Mém. de la soc. imp. de Cherbourg, t. XIII, 1867, p. 145, *ff.*).—Kraus et Millardet, Etude s. la mat. color. des Phycochromacées et des Diatomées (Mém. de la soc. des sc. nat. de Strasbourg, t. VI, 1868, p. 23, *ff.*).—Millardet, Sur la nature du pigment des

Fucoidées (Comptes rendus, t. LXVIII, 1869, p. 462, *ff.*).—Kraus, Z. Kenntn. d. Chlorophyllfarbstoffe, Stuttg., 1872.—Pringsheim, Ueber natürl. Chlorophyllmodif. u. d. Farbstoffe, d. Florideen (Monatsber. d. K. Acad. d. Wiss. Berlin, 1875, p. 749, *ff.*).—Sorby, On the characteristic coloring-matter of the red groups of algæ (Journal of the Linn. Soc. Vol. XV, 1875, p. 34, *ff.*).—Reinke, Beitrag. z. Kenntn. d. Phycoxanthins (Pringsheim's Jahrb., Bd. X, 1876, p. 399, *ff.*).—Nebelung, Spectrosk. Unters. Uber d. Farbst. einiger Süsswasseralgen (Bot. Zeitg., 1878, p. 369, *ff.*).

The coloring substances of algæ are all, so far as they occur as cell contents, joined with the plasma, very like chlorophyll, and are to be included among its modifications. That indeed a great number of the algæ possess true chlorophyll is a well known fact; while in others, on the other hand, a green coloring matter occurs which is distinct from true chlorophyll in its chemical as well as in its spectroscopic behavior. The coloring substances of algæ may be divided into two groups in respect to their behavior towards alcohol and water, those of the one being soluble in alcohol but in water insoluble, and those of the other soluble in water, but mostly not in alcohol.

The more exactly investigated are the following.

A	B
Soluble in alcohol, not in water.	*Soluble in water, not in alcohol.*
1. Floridia green (green).	4. Phykoërythrin (red).
2. Phykoxanthin (yellow).	5. Phykocyan (blue).
3. Diatomin (yellow-brown).	6. Palmellin (red).
	7. Phykophaein (brown).

1. *Floridia green* is extracted from the *Floridia* by means of alcohol, represents a variety of chlorophyll, and is really very like it.

2. *Phykoxanthin.* Yellow coloring matter in kelp and numerous fresh water algæ in connection with protoplasmic bodies. It is easily extracted from the former by means of 40 per cent alcohol which will not dissolve true chlorophyll. Evaporate the solution and the coloring matter remains behind a slimy, amorphous, brown mass. Alcoholic solutions of phykoxanthin are made blue green by acids but are not changed by alkalies.

3. *Diatomin* (endochrom). The yellow to brown-yellow coloring matter of the *Diatomaceæ*. It becomes greenish by application of acids and alkalies and with concentrated sulphuric acid a beautiful verdigris geeen. It consists of phykoxanthin and chlorophyll.

4. *Phykoërythrin* (*Floridia red*). The red coloring matter of the *Floridia* appears the same when dry, is soluble in water but not in alcohol or ether. The aqueous solution loses its color in the light. Alkalies color it pale olive green (almost colorless), acids restore the red color again. Concentrated sulphuric acid does not alter the aqueous extract.

5. *Phykocyan* (*Phykochrom*). In blue-green algæ. The blue-green or indigo blue coloring matter is soluble in water but not in alcohol, becomes yellowish brownish or yellow green with alkalies; with muriatic acid, orange red or smutty orange.

6. *Palmellin*. A red coloring matter of *Porphyridium* which is soluble in water and becomes blue with alkalies.

7. *Phykophaëin*. A brown coloring matter from the *Fucaceæ*, soluble in water and dilute alcohol, but insoluble in absolute alcohol, ether and benzole. It is intercalated in the protoplasmic grains in connection with chlorophyll and phykoxanthin. The aqueous solution is intense brown red but is not fluorescent. Absolute alcohol, cold, produces a cloudiness in the solution. By warming, the coloring matter is thrown down as a partly flaky brown precipitate. A like effect is produced by muriatic nitric and sulphuric acids. Concentrated alkalies bleach the solution somewhat.

The above described coloring substances commonly occur in connection with chlorophyll and its optical effect mixes, in the living plant, with that of chlorophyll.

Spectroscopic behavior. Some of the above named coloring substances of the algæ have been subjected to critical spectroscopical studies, while others are in this regard quite unknown. The following have been exactly tested spectroscopically.

Floridia-green and *Floridia-red* (Pringsheim).[206] The Phykoërythrin (Florida-red) shows a spectrum which possesses all

[206] Pringsheim in Monatsber. d. K. Acad., Berlin, 1875, pp. 749–754.

the essential marks of the chlorophyll spectrum (Fig. 150, constructed after Pringsheim's method *l. c.*, with Angströms scale for a standard; 0, 10, 20, etc., gives the optical concentration of the solution under investigation; the dotted line is the absorption curve of the phykoërythrin, the full line that of the Floridia-green). But the chlorophyll bands III, IV and IV*a* (see p. 413, *ff.*) appear to be considerably strengthened in the phykoërythrin, bands I and II being very much weakened while the bands in the blue and violet remain unchanged in their intensity. With some coloring substances which belong

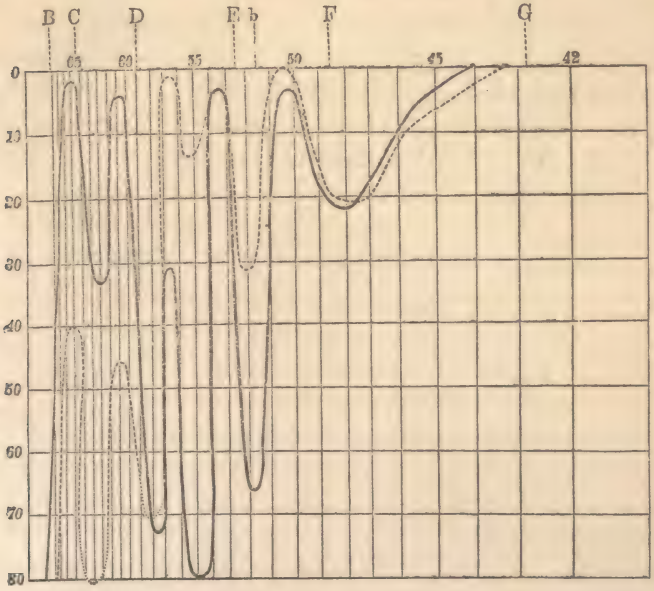

FIG. 150.

to the phykoërythrin group, there appear to be some minor differences in the weakening of bands I and II. But on the whole the maxima and minima of the absorption curve coincide with those of the absorption curve of chlorophyll.

The green alcoholic extract of the *Floridia* (Fig. 150) is spectroscopically somewhat different from chlorophyll. Its spectrum differs from that of chlorophyll by a slight weakening of bands I, II and III, and by a considerable strengthening of

band IV, and of the bands in the blue and violet, which in a medium optical concentration flow together to make an end absorption, and finally by a new maximum of absorption which includes the wave lengths 51 and 49 (unit = 0.00001 mm.).

By comparing the spectra with each other (Fig. 150) there is seen to be a very exact coincidence of the absorption maxima and minima, from which it becomes apparent that Floridia-red is a modification of Floridia-green, and not a direct modification of the phanerogamic chlorophyll (Pringsheim).

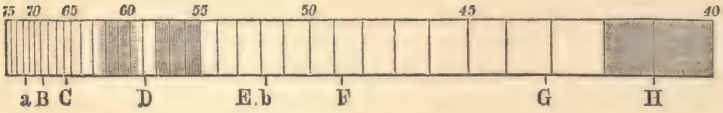

FIG. 151.

Phykocyan (Reinke). The clear blue, aqueous extract of *Oscillaria*, which has a red fluorescence, gives in a layer 15 cm. thick a spectrum with four absorption bands (Fig. 151 after Reinke), of which III is very weak. If the solution be boiled only the bands at F and H remain visible, likewise it loses its fluorescence.

If after the extraction of the phykocyan, the Oscillaria be again subjected to extraction by alcohol, and the filtrate shaken

FIG. 152.

up with benzole, phykoxanthin is retained in the alcohol as an amber yellow fluid. This gives a spectrum like that of chlorophyll (Fig. 152 after Reinke), with this difference that band II shows a not unimportant broadening towards the red end of the spectrum. It even divides sometimes into two bands; band III is also broadened towards the red side. Of the bands of the

second half of the spectrum, VI and VII coincide with the corresponding chlorophyll bands, IV and V differ from them[207].

Concerning the older spectroscopical investigations of the coloring matter of algæ, which were commonly made without specifying the optical concentration and have therefore only relative value, one may compare the above cited writings of Cohn, Kraus, Millardet, Rosanoff, and Askenasy.

It may be remarked by way of appendix that Nägeli[208] mentions two membrane coloring substances of algæ, viz., Gloeocapsin and Scytonemin, Gloeocapsin occurs in the membranes of Gloeocapsa and some other algæ, and is a red or blue coloring matter which is colored rose, red orange, or brown red by muriatic acid, and with potash lye blue or blue-violet. Scytonemin is a yellow or dark brown coloring matter in the walls of the *Phycromaceæ* which becomes verdigris green with muriatic acid, and with alkalies yellow, often almost gold yellow.

2. Fungi Coloring Matter.

Literature. Schröter, Ueber eininge durch Bacterien gebildete Fermente (Cohn's Beitrag. z.,Biol. d. Pfl., Bd. I, 1872, p. 109, *ff.*).—Klein in Quart. Journ. of Microsc. sc., 1875, p. 381, *ff.*—Sadebeck, Durch mikrosk. Organismen rothgef. Wasser (Verf. d. bot Ver. d. Prov. Brandenburg, Bd. XVII, 1876, p. 77, *f.*)—Cugini, Sulla materia colorante del Boletus luridus (Gazetta chimica, Vol. VII, 1877, p. 209, *ff.*).

In the group of fungi coloring substances of very different nature seem to occur in great numbers all of which have nothing whatever to do with chlorophyll. Alas! that these substances have not been at all studied. We can therefore give here only some altogether superficial statements.

First. Coloring matter frequently occurs in the *Schizomyceteæ*. The color producing bacteria show different colored pigments (red, yellow, green, blue, brown) of intense shades. They are insoluble in water, alcohol and ether.[209] Alcohol and

[207] Reinke in Pringsheim's Jahrb., Bd. X, p. 406, *ff.*

[208] Nägeli u. Schwendener, Mikr., p. 507.

[209] According to Sadebeck, *l. c.*, the red pigment of micrococcus is partly soluble in water.

ether remove the red from the bacteria pigment, potash solution makes it transparent. The red coloring matter of *Micrococcus* is according to Helm[210] aniline red. With muriatic acid it becomes rose colored, likewise with sulphuric acid (violet with the addition of more acid), with alkalies yellow. In this case acids restore the red color.

The coloring matter of *Agaricus atrotomentosus* dissolves in alcohol and acetic acid with rose red color, becomes yellow with alkalies and is insoluble in water and benzole.

According to Phipson aniline-like coloring matters occur likewise in *Boletus luridus* and *B. cyanescens*. Cugini controverts this and gives the following characteristics of the "acid-like" coloring matter of *Boletus luridus*. It is soluble in water and alcohol, acids color it a beautiful yellow (chromic acid, yellow brown), ammonia blue, potash-lye red. Ferric chloride gives it an intense green color.

Spectroscopically the fungi coloring substances, with the exception of the bacteria pigment, have not been investigated. The spectra have not the remotest likeness to those of chlorophyll coloring matter.

[210] O. Helm in Arch. f. Pharm., 1875, p. 19, *ff.*

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ERRATA, ETC.

Page 19, line 8, before *A* insert]. Page 32, line 20, for *J* read *T*. Page 43, Fig. 17 is printed upside down. Page 52, line 11, for *opposite* read 50*th*. Page 91, in Fig. 31, the lower middle figure should show a black center-stop instead of a central aperture. Page 92, line 7, for *it is hoped will soon be*, read *has lately been*. Page 108, line 4, for *cm* read *mm*. Page 109, line 6 from bottom, after *The* insert *dotted*. Pages 156–7 for Microscopical objects and preparations read Microscopic, etc. Page 158, 2nd paragraph, 10th line, read primordial *utricle*. Page 282, first line, read, "To prepare a potash solution of a definite concentration." Page 296, 2nd paragraph, 2nd line, read fuming for foaming. Page 307, 5th line from bottom, for Beal's read Beale's. Page 350, 9th line from top, read fall for falls.

Headline of section, p. 121, for I read 1. Headline p. 126, for B. read 3. Headline, p. 127, for III read IV. Headline, p. 128, for IV read V. Headline p. 133, for IV read VI. Headline p. 139, for V read VII. Headline p. 177, for Microscopical read Microscopic.

The metric system of weights and measures is used throughout in this work. The degrees of temperature are from the centigrade thermometer.

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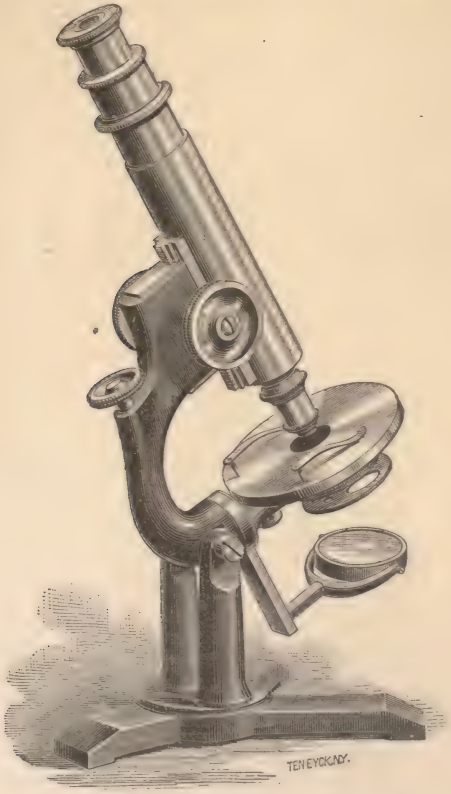

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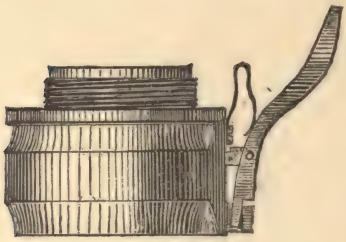

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